



# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 434

ON THE FAT TOLERANCE TEST

BY

GUNNAR ANGERVALL

---

*Accompanied Vol. 176*

GÖTEBORO 1966



# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 424

ON THE FAT TOLERANCE TEST

BY

GUNNAR ANGERVALL

*Accompanied Vol. 176*

---

GÖTEBORG 1964



# ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of *Nordiskt Medicinskt Arkiv* founded in 1869 by Axel Key. The first volume of *Acta Medica Scandinavica* is therefore numbered LII (52).

*The chief editors have been: Axel Key 1869—1900, C. G. Santesson 1901—1915, I. Holmgren 1916—1957 and Birger Strandell 1958 to date.*

*Acta Medica Scandinavica publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board. Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the Editor at the address below.*

*Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that, if accepted, it will not be republished in any other journal in the same or similar form, without the Editor's written consent.*

*Papers will be published in English, French or German at the authors' choice, followed by a summary in English.*

*The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left hand margin.*

*If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author. No paper shall exceed 24 printed pages.*

## Subscription

*The annual subscription to the journal, covering two volumes each of 6 numbers, is 140 Sw. crowns or U.S. \$ 27.25 including postage in the Scandinavian countries and in Holland 120 Sw. crowns.*

*Address for subscriptions and all communications*  
ACTA MEDICA SCANDINAVICA  
P O Box 2052 Stockholm 2

---

*Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number*

# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 424

FROM MEDICAL DEPARTMENTS I (HEAD: L. WERKÖ, M.D.) AND III (HEAD: T. LINQVIST, M.D.),  
SÄHLGRENKA Sjukhuset GÖTEBORG, SWEDEN

## ON THE FAT TOLERANCE TEST

BY

GUNNAR ANGERVALL

GÖTEBORG 1964

Translated  
by  
KLAS MAGNUS LINDSKOG

ÖSTERBOM 1944  
ELANDERS BOKTRYCKERI AKTIEBOLAG

# CONTENTS

	Page
	5
	7
I Introduction	7
II Survey of the Literature	8
The composition of the fat meal	8
Time of administration	9
The diet prior to fat loading	10
Work and fat tolerance	10
Reproducibility of fat tolerance	12
Serum lipid changes after fat loading	13
Age and fat tolerance	13
Body weight and fat tolerance	18
Atherosclerosis and fat tolerance	15
III Clinical materials	18
Classification	20
Controls	21
Hypercholesterolemia	21
Hypoparathyroidism	21
Vernixglyceridemia noncholesterolemia with atherosclerosis	24
Lecithemia	26
IV Methods	26
Turbidity determination	26
Extraction of lipids	26
Cholesterol determination	27
Phospholipid determination	27
Glyceride glycerol determination	29
The fat tolerance test	29
V Duplicate fat tolerance test	29
Turbidity	29
Glyceride glycerol	29
Cholesterol	29
Phospholipids	31
Assumptions between the glyceride-glycerol level of fasting serum and its cholesterol, phospholipid and viscosity	31
Assessment	31
VI Frequency analysis of changes in serum lipid factors in the fat tolerance test	37

Translated  
by  
KLAS MAGNUS LINDSKOG

GÖTEBORG 1944  
FLANDERS BOKTRYCKERI AKTIEBOLAG

## INTRODUCTION

At Medical Department I Sahl  
grenska sjukhuset Gothenburg fat  
tolerance tests — using a standard  
meal of 200 ml double cream (40 %  
butter fat content) — have been  
carried out routinely for the past  
several years, involving turbidity meas-  
urements among other estimations.  
The serum turbidity as a rule attained  
a maximum 4 hours after the fat meal  
an interval agreeing well with the  
reported responses to similar fat meals.  
After 7 hours the turbidity had nearly  
returned to its original value. In 1959  
a method for estimation of neutral  
fat as glyceride-glycerol was published  
by CARLSON & WADSTRÖM which esti-  
mates neutral fats directly unlike  
previous methods which estimated  
neutral fats indirectly as total lipids  
or total fatty acid less the correspond-  
ing cholesterol and phospholipid com-  
pounds and free fatty acids. The former  
method was immediately adopted with  
the object of analyzing the serum  
glyceride-glycerol changes in response  
to fat meal.

Although the majority of workers  
has maintained that the turbidity  
and neutral fat content of serum  
are associated no attempt has been  
made to establish the nature of this  
association. This would provide a  
means for assessing the value of the  
serum turbidity measurement which

by virtue of its simplicity so often  
is made.

As has been the case in a number of  
previous publications, the present in-  
vestigation included estimation of the  
cholesterol and phospholipid levels  
of serum in order to classify the patients  
in the conventional manner and to  
study the relations of these lipids to  
glyceride-glycerol in serum at the fat  
tolerance test.

The authors who have studied the  
reproducibility of fat tolerance tests  
have merely stated in general terms  
that in their opinion, the alimentary  
lipaemia induced by the fat meal was  
constant in the individual none of  
them published any numerical data in  
support of this contention.

In most published investigations  
involving the use of the fat tolerance  
test for studying alimentary lipaemia  
the aim has been to compare and  
contrast a group of patients with  
coronary heart disease and a control  
group. Opinions have been expressed  
about the value of such comparisons.  
The fat tolerance of the controls has  
in most cases proved greater than  
that of the patients. FÖRDMAN, Z.  
REINFIELD & CHESNIN (1934) and  
LILJO JÖRNER, & REINHOLD (1956)  
have pointed out the fact verified by  
this writer that hypercholesterolaemia  
itself is a factor that tends to prolong

VII	The fat tolerance test in various groups of subjects	Page 39
	Serum lipids and turbidity in the control groups	30
	Serum lipid 4 hours after and immediately before the fat meal	40
	Serum lipids 7 hours after and immediately before the fat meal	44
	Serum lipids 7 and 4 hours after the fat meal	47
	Comment	51
VIII	Glyceride glycerol level and turbidity of serum of its subnatant and its chylomicron phase	56
	Glyceride-glycerol of whole fasting serum and its subnatant	50
	Turbidity and glyceride glycerol of whole fasting serum and its subnatant	57
	Glyceride-glycerol of whole serum and its subnatant 4 hours after the fat meal	60
	Turbidity and glyceride-glycerol of whole 4-hour serum and its subnatant	62
	Glyceride glycerol of whole serum and its subnatant 7 hours after the fat meal	63
	Turbidity and glyceride glycerol of whole 7 hour serum	64
IX	Effect of dietic changes on the fat tolerance test	66
	Definition of diets	66
	Technique for repeated fat tolerance tests	66
	Fat tolerance test on ordinary diet and on a high poly-unsaturated fats diet	68
	Fat tolerance test on ordinary diet and on a low-saturated fat diet	69
	Comment	70
X	General discussion	73
	Reproducibility	73
	The individual's response to the fat meal	74
	Fat tolerance tests in different categories of subjects	74
	Serum and its subnatant	75
	Fat tolerance tests on different diet	76
XI	General summary	78
	Acknowledgments	80
	References	81

## CHAPTER II

### SURVEY OF THE LITERATURE

As long ago as 1622 ANELLIUS discovered that food absorbed by the small intestine was poured out into the mesenteric lymphatics and made these vessels visible by their milky appearance. In 1680 PRIGOUR succeeded in tracing these vessels to the great veins of the neck, and in 1693 BORD found that the blood serum itself was made milky after a meal. Rather more than a century later in 1774 HENSON demonstrated that this milky fluid contained fat and also that under the microscope it showed exceedingly fine particles. And in 1887 EDWARDS making his observations in a dark field microscope first described these particles as chylomicrons. (After GANG & FREN 1924)

NEUMANN (1907) seems to have pioneered the study (by chylomicron counts) of quantitative lipid changes in the blood following a fat load.

Over the years more and more refined methods for determination of fat in blood have been developed. The ensuing survey of the literature will be confined mainly to publications relevant to the present investigation namely those dealing with fat loading by the oral route of non radioactive fat

had to have his own control series. Apparently the most common practice has been to administer the fat as a mixed meal implying that the patient is loaded with a roughly estimated amount of fat together with carbohydrates and protein in the form of say a breakfast of bacon and eggs cream, bread and butter etc. Whenever fat only has been given, it has generally consisted of cream or olive oil.

The effect of carbohydrates upon the lipaemia attending fat meals in dogs was studied by BAXG (1918). When bread was substituted for the meat in the meals given the dogs he occasionally observed that the degree of alimentary lipaemia became lower than after fat plus meat. ALBRINK & MAY (1936) gave human subjects glucose 1 hour and  $\frac{1}{2}$  hour before as well as  $1\frac{1}{2}$  hours after a fat meal finding that this reduced or even eliminated the serum triglyceride rise. (See also MAY & ALBRINK 1936) BEKOWITZ, LIKOFF & SPRITZER (1930) reported that a flatter triglyceride curve resulted when glucose was given simultaneously with  $^{125}$ I labelled trolein than when no glucose was given.

Little is known about the effect of protein on the lipaemia induced by fat. In BAXG's experiments on dogs meals of fat plus meat frequently

#### Fat Loading

The composition of the fat meal has varied widely and each investigator



the alimentary lipaemia induced by a fat meal. In the majority of published investigations serum turbidity was measured, the higher turbidity in atherosclerosis usually being interpreted as higher lipaemia, although it could also represent a higher visible fat content, i.e. chylomicron fat. In the present investigation, therefore, particular attention was paid to whether the estimations of chylomicron lipids and turbidity disclosed any difference between subjects with and without atherosclerosis.

There exist no generally accepted definitions of the concepts hypercholesterolaemia and hyperlipaemia. In the present investigation a hyperlipaemic will apply to a subject who on at least two occasions, has fasting serum with a glycerido-glycerol level of at least 2 mmol/l as well as a turbidity exceeding 0.10. In other words the writer wishes to retain the concept of serum *laetescence*, a laetescent serum being one with a turbidity exceeding 0.10. Normoglyceridaemias have in turn been classified into normocholesterolaemias and hypercholesterolaemias, the cholesterol level of the latter a fasting serum on at least two occasions exceeding 300 mg.

The terms atherosclerotics and non atherosclerotics are used in the literature with somewhat varying connotations. Here atherosclerosis will have the following implications:

1 Classical angina pectoris and myocardial infarction

2 Angiographic manifestations of atherosclerosis

3 Intermittent claudication with positive oscillometry

The value of the fat tolerance test should be judged by whether it causes say groups with and without clinical atherosclerosis with similar lipid factors of fasting serum to exhibit any differences after a fat meal.

#### Aims of the investigation

To assess the reproducibility especially in hypercholesterolaemias and hyperlipaemias of the alimentary lipaemia induced by a standard fat meal in terms of serum turbidity, glycerido-glycerol, cholesterol and phospholipids determined both immediately before and 4 hours after the test meal.

To study these serum lipid factors before as well as 4 and 7 hours after a standard fat meal in (a) controls, (b) hypercholesterolaemias and hyperlipaemias, classified into subgroups with and without clinical atherosclerosis and (c) normoglyceridaemic normocholesterolaemias with clinical atherosclerosis (males only).

To study the distribution of serum neutral fats on the chylomicron phase and subnatant at the fat tolerance test and in so doing to analyze the association between neutral fats and turbidity.

To compare the same subjects' alimentary lipaemias in response to the standard fat meal when he is on a diet containing predominantly saturated fats and when he is on a diet containing predominantly polyunsaturated fats.

no change in fat tolerance when the fat content of the diet was raised from 40 to 54 per cent for three weeks. BROUKE-STEWART & BLACKBURN (1958) reported that whereas 11 patients on a fat restricted (less than 30 %) diet had "high curves" 12 other patients on an unrestricted diet included 2 with low curves and 10 with "high curves". ALBRINK & NEUWIRTH (1960) stated that the alimentary rise was reduced after 7 days of starvation. Pointing out that the Bantu the Cape Coloured and the European populations of Cape Town consume 17, 45 and 60 per cent respectively of their total calories as fat, BOUCHNER & BROUKE-STEWART (1961) could discern no difference between these groups in their tolerance to a test meal of fat. The authors did not specify the calorie intake or the composition of the fat in the diet. The significance of fats of the polyunsaturated type was demonstrated by BROUKE-STEWART & BLACKBURN (1958) whose patients with ischaemic heart disease and "high curves" after merely a week on maize oil had their fat tolerance altered so that their test curves became indistinguishable from those of the controls. It has been observed that the shape of the fat tolerance curves (turbidity) is dependent upon previous dietary fat intake (JONES & DOBRILOVIC 1963).

Work and fat tolerance. VASEY (1931) stated that fat loading increased the serum lipid level of normal subjects at rest (in bed) by 4 per cent and the maximum was attained after 4 hours while the corresponding figures were 31 per cent and 3 hours for a

group of subjects at work (under graduates and nurses). GAGE & FINE (1944) recorded higher chylomicron counts after fat loading in persons at work than in persons at rest unlike MARDER *et al* (1953) who reported the opposite result. BARBITT (1950) maintained that limited physical activity seemed a relatively unimportant factor. The fat tolerance curves of his bed and ambulant patients showed no obvious optical density differences. BILLIMORIA *et al* (1959) found that alimentary lipaemia occurred earlier in exercising than in resting subjects. McDONALD & FOLLESTON (1960) found that the increase in plasma turbidity after a fat rich meal was lower in ambulatory patients than in patients confined to bed.

COHEN & GOLDBERG (1960) studied the lipaemia induced by fat loading in healthy undergraduates both during rest and during exercise (a 0.4 kilometre walk in about 1½ hours) noting that the serum turbidity was significantly lower during exercise. NIKETLA & HOVTTINEN (1962) had similar results by estimating neutral fat in serum according to VAN HANDEL & ZILVERSMITH (1957).

Some other factors have been claimed to affect the fat tolerance. For example one cigarette per hour caused the chylomicron count to rise in a group of young subjects but not in two elderly subjects (MARDER *et al* 1953). When habitual smokers were tested with a fat meal the postprandial rise in serum neutral fat was lower in the smoking than in the non-smoking group (HOVTTINEN & ILARINEN 1963).

produced a higher degree of lipaemia than fat alone although meat alone did not give lipaemia BIKO & HEOK SCHER (1924) found that when meat was given together with fat the maximum of alimentary lipaemia occurred earlier and disappeared faster than when fat was given alone SULLIVAN (1962) found a prolongation and intensification of lipaemia in 4 out of 8 human subjects after protein and fat ingestion as compared with fat ingestion alone

Usually the fat dose has fallen in the range from 0.5 to 4 g per kg body weight or a standard meal as a rule containing 50 to 100 g of fat (as much as 204 g by POMERANKE BEINFELD & CHESNIN 1954) has been given No association has been demonstrated between body weight and the duration of lipaemia after a standard fat meal (BARRITT 1956) Very obese subjects exhibited excessive and prolonged lipaemia following a fat load (POMERANKE BEINFELD & CHESNIN 1954) MAN & GILDEA (1932) gave up to 4 g of fat per kg body weight a dose nauseating many patients but demonstrated no association between the degrees of lipaemia and nausea MORETON (1950) and KINGSBURY MORGAN & SHERVINGTON (1960) on the other hand maintained that nausea was accompanied by slowing of peristalsis and consequently by altered fat absorption in the digestive tract

*Time of administration* As a rule meals have been given in the morning after 12 to 14 hours fasting A departure from this rule was made by KINGSBURY MORGAN & SHERVINGTON

(1960) who gave a high-carbohydrate low fat meal 2 or 3 hours before the fat meal itself the reason given by the authors being that the subjects would then be less nauseated by the fat load

BOHM GERNANDT & HOLMGREN (1941) analyzed diurnal variations in the chylomicron count after fat loading and found that it attained a maximum when the fat load was administered during the night

*The diet prior to fat loading* has a decisive effect upon the results As long ago as 1924 GAGE & FISH found that fat loading was attended by lower chylomicron counts after 144 hours fasting than after 12 hours fasting BLITZ (1925) reported that the elevation after fat loading was accentuated when the subject had been merely a few days on a Petró diet (200–250 g of fat daily) but this alimentary accentuation in due course disappeared and the chylomicron count rose returned to initial size Although BLITZ discussed adaptation as a possible explanation of this phenomenon he was more inclined to ascribe it to variations in the rate of fat removal from the blood The fat tolerance was improved by 6 weeks isocaloric fat restriction to less than 20 g daily (POMERANKE BEINFELD & CHESNIN 1954) Under metabolic ward conditions HAVEL (1957) obtained appreciable fat tolerance differences in healthy subjects on an isocaloric diet when their daily fat intake per kg body weight was varied from 0.1 to 2.0 g with the highest fat tolerance after the lowest intake

Conversely HORLICK (1957) found

no change in fat tolerance when the fat content of the diet was raised from 40 to 54 per cent for three weeks BROSTE-STEWART & BLACKBURN (1938) reported that whereas of 11 patients on a fat restricted (less than 30 , ) diet had high curves 12 other patients on an unrestricted diet included with low curves and 10 with "high curves" ALBINK & NEUWIRTH (1960) stated that the alimentary rise was reduced after 10 to 7 days of starvation. Pointing out that the Bantu the (ape) coloured and the European populations of Cape Town consume 1 43 and 60 per cent respectively of their total calories as fat BOTCHIK & BROSTE-STEWART (1961) could discern no difference between these groups in the tolerance to a test meal of fat. The authors did not specify the caloric intake or the composition of the fat in the diet. The significance of fats of the polyunsaturated type was demonstrated by BROSTE-STEWART & BLACKBURN (1938) whose patients with ischaemic heart disease and high curves after merely a week on mace oil had their fat tolerance altered so that their test curves became indistinguishable from those of the control. It has been observed that the shape of the fat tolerance curves (turbidity) is dependent upon previous dietary fat intake (JOYNS & DOBRILOVIC 1962).

Work on fat tolerance VIKARI (1931) stated that fat loading increased the serum lipid level of normal subjects at rest (in bed) by 42 per cent and the maximum was attained after 4 hours while the corresponding figures were 14 per cent and 3 hours for a

group of subjects at work (under graduates and nurses). GANZ & FISH (1924) recorded higher chylomicron counts after fat loading in persons at work than in persons at rest, unlike MARDER *et al* (1952) who reported the opposite result. BARNITT (1936) maintained that limited physical activity seemed a relatively unimportant factor: the fat tolerance curves of his bed and ambulant patients showed no obvious optical density differences. BILLIMO *et al* (1950) found that alimentary lipaemia occurred earlier in exercising than in resting subjects. McDONALD & FULLERTON (1960) found that the increase in plasma turbidity after a fat rich meal was lower in ambulatory patients than in patients confined to bed.

CORRY & GOLDBERG (1960) studied the lipaemia induced by fat loading in healthy undergraduates both during rest and during exercise (a 9.4 kilometre walk in about 2 1/2 hours) noting that the serum turbidity was significantly lower during exercise. VIKARI & KONTTINEN (1962) had a similar result by estimating neutral fat in serum according to VAN HANDEL & ZILVERSMITH (1937).

Some other factors have been claimed to affect the fat tolerance. For example one cigarette per hour caused the chylomicron count to rise in a group of young subjects but not in two elderly subjects (MARDER *et al* 1952). When habitual smokers were tested with a fat meal the postprandial rise in serum neutral fat was lower in the smoking than in the non-smoking group (KONTTINEN & RAJALAMMI 1962).

"Stress" was responsible for a peak in the chylomicron curve (GAGE & FISH 1924)

*Reproducibility of fat tolerance* Some workers have studied variations in fat tolerance NISSEN (1931) for example expressed the opinion that a fat load of 1 g per kg body weight consistently gave rise to the same fat tolerance curve in the same normal subject MORETON (1950) and OSMON /INN & WHARTON (1957) concurring made similar observations even when the interval between consecutive fat tolerance tests was up to 6 months Slight variations however have been reported by other workers (MESSINGER & POROSOWSKA 1953 BARRITT 1956) Performing repeated fat tolerance tests in 15 patients with ischaemic heart disease and in 10 controls BROVTE STEWART & BLACKBURN (1958) found considerable variability in response to the same fat load although those who exhibited a high curve continued to do so and vice versa The fat load had the form of a breakfast of bacon and eggs etc and its fat content was less well defined than the fat meals given by the other authors mentioned here DEYBROUVOH (1963) found that duplicate fat tolerance tests (70 g fat as a mixed meal) were consistent (triglycerides according to VAN HANDEL & SILVERSMITH) JONES & DOBRILOVIC (1963) performed 62 fat tolerance tests (breakfast with 30 g fat) in 7 patients and found that the total fatty acid content of the chylomicron fraction did vary with the optical density and that the shape of the fat tolerance curve was dependent upon the previous

dietary fat intake as well as on the quality of the test fat

#### Serum Lipid Changes After Fat Loading

*Chylomicron counting* seems to represent the very first attempt at quantitative blood fat estimation (NEUMANN 1907) and the method remained in use well into the 1950's (e.g. MARDER *et al* 1952 GRÖNER & HILDEY 1955)

Different fats give rise to specific types of chylomicrons (SWANK & LEVY 1952) Triolein gave large distinctive spherical chylomicrons while those seen after tristearin and cream vary in shape and are comparatively small The chylomicrons are small (about  $0.5 \mu$ ) at the beginning and end of the fat tolerance curve but larger (about  $1 \mu$ ) at maximal lipaemia (GAGE & FISH 1924) Even though most of the blood fat increase occurs in the form of chylomicrons (GRÖNER & HILDEY 1953) the chylomicron count would seem to be a poor estimate of the serum lipid increase after a fat meal

*Turbidity* Another way of estimating visible lipids in serum is to measure its turbidity either nephelometrically or spectrophotometrically The former technique directly measures the turbidity as reflected light requiring special instruments and standard solutions — BaSO<sub>4</sub> suspension in glycerol — it is the more complex of the two (MORETON 1950 MARDER *et al* 1950 MESSINGER & POROSOWSKA 1953) The latter technique is indirect and measures the degree of light absorption by optically active particles

Serum turbidity has in most cases been measured spectrophotometrically at wavelength 680 m $\mu$  with variations in the range 810—700 m $\mu$ .

Particles with diameters more than one-quarter the wavelength of visible light (or 0.1  $\mu$ ) deflect and scatter incident rays of light and give the serum the appearance of turbidity or lipaemia (ARBUCK & KUSHEL 1948). Neutral fat is the only lipid consistently associated with serum lactescence, and the soluble neutral fat of lactescent serum was found to be between the limits 10 and 30 mEq/l neutral fatty acids when total fats varied from 16 to 478 mEq/l (ALBUCK, MAX & PETERS 1954).

The turbidity and neutral fat content of serum were as a rule well correlated in BARSTY'S (1956) patients. ELWATER & SCHETTLER (1938) found closely parallel turbidity and neutral fat values along the ascending portion of the fat tolerance curve after 1 meal call but the two diverged along the descending portion this deviation was less marked after coconut oil or butter (DREW, LIPP & WILSON 1957) remarked that serum turbidity was correlated with rise in total lipids in their studies on the tolerance to 1 g of fat per kg body weight. JONES & DONALDSON (1963) is of the opinion that optical density provides a fairly reliable indication of the postprandial changes in chylomicron fatty acid concentration.

*Cholesterol* The majority of investigations have shown that the serum cholesterol level remained constant or was negligibly changed after a fat load

(BLOOR, 1916; BLIX 1945; HIRSCH & CARONARO 1950; POMERANCE, BIRNFIELD & CHESSEX 1954; ALBUCK & MAX 1956). Not so HILLER *et al* (1934), who recorded an average increase of 5 per cent and BAUM (1940) with a mean cholesterol rise of 20 mg % after a fat meal. HAVEL (1937) obtaining no rise in men claimed that 7 of 8 women showed an increase of at least 15 mg % in very low density lipoproteins. BLOTTEN (1935) stated there was no rise in thin persons but a noticeable rise in fat persons. On the other hand VIKKILI & KORTTIWEX (1962) found a significant decrease 6 hours after loading with 55 g butter fat in healthy soldiers.

KINGSBURY & MORRIS (1960) reported different cholesterol responses after 16 different oils, while some of them induced a serum cholesterol (and phospholipid) rise of approximately 20 mg %, others instead induced a reduction.

*Phospholipids* There seems to be substantial agreement that fat loading raises the amount of serum phospholipids. MAX & GILDSA (1932) noted a slight phospholipid increase 3 hours after 60 g of fat but an 18 per cent rise after between 3 and 4 g fat per kg body weight. POMERANCE, BIRNFIELD & CHESSEX (1954) recorded phospholipid increases between 28 and 94 mg % five hours after a fat meal the rise being lowest in the group of extremely obese male individuals (40—40 years) with 29 mg % and normal males with normal lipids (20—20 years) with 33 mg % and highest — 81 mg % — among males over 70 years with

clinical evidence of atherosclerosis" the group showing the highest alimentary lipaemia ALBRINK & MAY (1956) on the other hand obtained no phospholipid increase after a load of 60 g fat in a mixed meal Recently other authors have also observed in crease of phospholipids in similar trials (TALBOTT & KEATING 1962 SKIBBE & HAUGE 1963)

HAVEL (1957) recorded increases of both high density and low density lipoprotein phospholipids. A delayed fall was due to the persistence of the increased concentration of high density lipoprotein phospholipids after the visible lipaemia had disappeared

*Total fat neutral fat* Several previously quoted authors have estimated total fats at fat tolerance tests (HERZSTEIN *et al* 1953 BARRITT 1956 OSWICK ZINN & WHARTON 1957) Others have estimated total fatty acids (POKORANZ, BEINFELD & CHESIN 1954 HORLICK 1956 1957) Neutral fat has been calculated as total fat or total fatty acids less the corresponding cholesterol and phospholipid compounds and free fatty acids (LAK & ALBRINK 1956 BARRITT 1956 EGGSTEIN & SCHETTLER, 1958 TALBOTT & KEATING 1962 SKIBBE & HAUGE 1963)

Direct estimation of neutral fat has been enabled by chromatographic separation This technique was introduced for clinical use by CARLSON & WADSTRÖM (1950) A similar method for neutral fat was worked out by VAN HANDEL & ZILVERSMITH (1957) With this method NIKKILÄ & KONTTINEN determined neutral fat in serum in 20

healthy soldiers after a breakfast with 55 g fat Fasting neutral fat in serum was 98 mg% and increased 4 hours after the fat load to 175 mg% DENBOROUGH (1963) gave 70 g fat as a mixed meal to controls Neutral fat in fasting serum was 110 mg% and increased to about 240 mg% 3½ hours after the fat meal The author found a correlation between the fasting and 3½ hours values as well as between the fasting and the 7½ hours values Also between the maximal and fasting triglyceride values there was a significant correlation

*Age and fat tolerance* Investigations into the association between age and fat tolerance have had divergent results. BECKER, MEIER & NECHLES (1949 1950) stated that the chylomicron count after a fat meal rose more in a group of 30 subjects over 50 (mean age 70 years) than in a group of 30 subjects under 50 (mean age 18 years) MARDER *et al* (1952) reported similar results of turbidity measurements Despite using the same chylomicron counting principles (FRAZER & STEWART 1937) as BECKER, MEIER & NECHLES, GRÖNER & HILDES (1953) were unable to verify their findings but found instead that older subjects (over 50 years) had a significantly lower chylomicron count in response to fat loading than younger subjects Notably however while the test meal consisted of 0.5 g margarine per kg body weight plus tea and bread in the two former investigations it was a standard meal of 150 ml cream with a butter fat content of 13 per cent in the latter study In both the control group and

the coronary disease group studied by SCHWARTZ, WOLDOW & DUXMORE (1952) the turbidity of serum after a fat load was higher in subjects over 40 years of age than in subjects under 40.

HERZSTEIN WANG & ADLERSBERG (1933) determined total fats after fat loading with 1 g fat (as 40 per cent cream) per kg body weight obtaining similar increases after hours in young (mean age 24.9 years) and old (mean age 66 years) subjects without metabolic disease or evidence of atherosclerosis. After 6 hours the total fats had returned to their initial level in the young group but remained enhanced in the old group. POMERANKE, BENEFIELD & CHESNIK (1954) found an elevated and prolonged fat tolerance curve (total fatty acids) in 6 males over 70 years of age with evidence of atherosclerosis, as compared with corresponding curves for other males between 20 and 40 years of age. The turbidity measurements and total fat estimations in BARRITT's (1956) investigation disclosed no significant correlation between age and the degree of lipaemia induced by a fat meal. But he remarked that young men showed a tendency to greater lipaemia. Nor did BOUCHIER & BROXTON-STEWART (1961) discover any turbidity difference between subjects over and under 40 years of age in conjunction with fat tolerance tests. BROWN HESLIN & DOYLE (1961) found that after a fat meal healthy middle-aged men 38-63 years of age cleared fat in serum more slowly than healthy male students.

*Body weight and fat tolerance* BARRITT (1956) observed no significant correlation between body weight and the duration of lipaemia in response to a standard fat meal. Similarly BROXTON-STEWART & BLACKBURN (1958) were unable to find any association between skinfold thickness and fat tolerance. Conversely TANWHAUSER (1947) stated that postprandial lipaemia might persist for unusually long periods in obese persons. The latter finding is in agreement with those of POMERANKE, BENEFIELD & CHESNIK (1954) who tested the fat tolerance of extremely obese male individuals (20-40 years) and found that their lipaemia (total fatty acids) was considerably prolonged but not elevated compared with that in normal males of corresponding age. The fat tolerance rose appreciably after weight reduction had been enforced.

*Atherosclerosis and fat tolerance.* MORRISON (1947, 1948, 1950) postulated that a relationship existed between chylomicrons and atherosclerosis. So did BROOKER, MEYER & NECHLES (1950) against the background of the longer and higher chylomicron curve they observed in old (over 50 years) than in young (under 50 years) subjects. SCHWARTZ, WOLDOW & DUXMORE (1952) reported that the turbidity of serum was greater in patients given a fat meal 1 to 8 weeks after onset of myocardial infarction than it was in the control group (convalescents after acute infections and minor surgical interventions) both before as well as 3 and 6 hours after a meal of cream and carbohydrates.

WOLDOW CHAPMAN & EVANS (1954)



tested the fat tolerance (standard cream meal) of 21 patients with evidence of coronary disease and noted that the lipaemia in this group was increased in both degree and duration compared with that in a control group. However, while the former group had a mean age of 55 years, the mean age of the latter was only 31 years.

POMERANZE, BETTSFIELD & CHESTNY (1954) reported that hypercholesterolaemics without other evidence of disease (20-40 years) and older males (over 70 years) with atherosclerosis showed a response to fat loading in the form of higher and longer lipaemia (total fatty acids) by comparison with young men (20-40 years) with normal blood lipids. BARRITT (1956) reported significantly higher fasting values for both turbidity and total lipids in men with coronary artery disease than in controls. The greatest difference was observed 5 and 7 hours after a load of 60 g fat. After a meal of 0.6 g heavy cream per lb body weight, KUO, JOYNER & REINHOLD (1956) recorded higher values at the 5th than at the 3rd hour in 6 of 7 hyperlipaemics, 10 of 12 hypercholesterolaemics, 10 of 15 atherosclerotic subjects (coronary heart

disease) and 2 of 10 controls. BRONTE STEWART & BLACKBURN (1958) 5½ and 7 hours after a 100 g fat breakfast found significantly higher serum turbidity in patients with ischaemic heart disease than in controls (schizophrenics) but a marked overlap between the groups existed. MITCHELL & BRONTE STEWART (1959) made similar observations when they compared turbidities in patients with ischaemic heart disease and in age-matched controls (convalescents) after a 75 g fat breakfast. Similar results were reported by BOUCHIER & BRONTE-STEWART (1961). HORLICK (1956, 1957) found that the total fatty acid level in cases of coronary atherosclerosis admittedly remained elevated longer than in the controls (young healthy males) but that a high fat test meal failed to uncover or exaggerate lipid metabolic defects in individuals with atherosclerosis.

Summing up, it may be stated that when hypercholesterolaemics and subjects with clinical atherosclerosis are subjected to fat tolerance tests, their fat tolerance curve will generally be higher and longer than in controls.

## CHAPTER III

### CLINICAL MATERIALS

Over the years 1959 through 1962 the subjects for the present investigation were selected at Medical Departments I and III and the Orthopaedic Department, Sahlgrenska Sjukhuset, Gothenburg. In addition the controls included some healthy subjects.

#### *Classification*

The groups of subjects may be classified as follows (see Table 1)

A Groups in which the individual was studied by repeating the fat tolerance test once or twice

1 The subject was on the same diet when two fat tolerance tests were performed, these observations were also used for assessing the reproducibility (Cf. Table 10 p 30)

The cholesterol phospholipid and glyceride-glycerol levels and turbidity were determined in serum specimens taken immediately before and 4 hours after a standard fat meal.

It was ensured that the patients were on the same diet preceding the duplicate fat tolerance tests and were free from infections or other diseases. The duplicate fat tolerance tests were performed on 45 in-patients and 9 out-patients, the interval between tests being  $8 \pm 0.3$  days in 51 cases and from 21 to 142 days in the remaining 6 cases. The age of the subjects ranged

from 25 to 68 years and averaged 47 years. 31 were up to and 26 over 50 years of age. Both these age groups included similar proportions of hyperlipaemic and hypercholesterolaemics

2. The subject was put on a different diet at least 6 weeks prior to the repeated fat tolerance test and the following comparisons were made.

The response to the fat meal of subjects on a diet with mainly saturated fats was compared with that of the same subjects on a diet with mainly poly-unsaturated fats. The intention was not to disclose any reductions in serum lipids or in the turbidity but rather to compare the courses of the responses to the standard fat meal on these diets. In 32 cases this comparison could be made between sera before and 4 hours after the fat meal and in 15 cases before as well as 4 and 7 hours after the fat meal.

The change from the subject's ordinary diet with saturated fats to one with poly unsaturated fats was usually made in two stages. The first stage was restriction of saturated fats. Comparisons could in 13 cases be made between the response on ordinary diet and that on a diet with restriction of saturated fats

In only 6 cases the fat test meal was given on ordinary diet on diet with restriction of saturated fats and on the

tested the fat tolerance (standard cream meal) of 21 patients with evidence of coronary disease and noted that the lipaemia in this group was increased in both degree and duration compared with that in a control group. However while the former group had a mean age of 55 years, the mean age of the latter was only 31 years.

POMERANZE, BEINFELD & CHESIN (1954) reported that hypercholesterolaemias without other evidence of disease (20-40 years) and older males (over 70 years) with atherosclerosis showed a response to fat loading in the form of higher and longer lipaemia (total fatty acids) by comparison with young men (20-40 years) with normal blood lipids. BARRITT (1956) reported significantly higher fasting values for both turbidity and total lipids in men with coronary artery disease than in controls. The greatest difference was observed 5 and 7 hours after a load of 80 g fat. After a meal of 0.6 g heavy cream per lb body weight. LYO, JOYNER & REINHOLD (1956) recorded higher values at the 5th than at the 3rd hour in 6 of 7 hyperlipaemics, 10 of 12 hypercholesterolaemics, 10 of 15 atherosclerotic subjects (coronary heart

disease) and 2 of 10 controls. BRONTE STEWART & BLACKBURN (1958) 5<sup>1</sup>/<sub>2</sub> and 7 hours after a 100 g fat breakfast found significantly higher serum turbidity in patients with ischaemic heart disease than in controls (schizophrenics) but a marked overlap between the groups existed. MITCHELL & BRONTE STEWART (1959) made similar observations when they compared turbidities in patients with ischaemic heart disease and in age-matched controls (convalescents) after a 75 g fat breakfast. Similar results were reported by BOUCHIER & BRONTE-STEWART (1961). HORLICK (1956, 1957) found that the total fatty acid level in cases of coronary atherosclerosis admittedly remained elevated longer than in the controls (young healthy males) but that a high fat test meal failed to uncover or exaggerate lipid metabolic defects in individuals with atherosclerosis.

Summing up it may be stated that when hypercholesterolaemics and subjects with clinical atherosclerosis are subjected to fat tolerance tests their fat tolerance curve will generally be higher and longer than in controls.



TABLE 1 Survey of clinical Series

Fat tolerance test group	Controls		Hypercholesterolaemia		Hyperlipaemia		Normochole normoglyc		Total	
			0 ath.	+ath.	0 th.	+ th.	normoglyc	+ath		
A Individual studies										
1 duplicate	♂	2		0		12		3	29	57
	♀	1		21		5		1	28	
2. repeated on different diets										
a. N and PU	♂	—		3	6	0	—		17	3
S-S	♀	—		4	1	3	—		15	
N and PU	♂	—					—		8	13
S-S-S	♀	—	3	1	0	3	—			
b N and FR	♂	1	1	0	2	3	1			13
S-S	♀	—		1	0	3	—		6	
c N FR and PU	♂	—	1	0	0	2	—		3	6
	♀	—	3	0	0	1	—		3	
B Group studies										
1 S-S-series	♂	4	11	28	14	8	13	116	10	
	♀	36	36	18	6	8	—	94		
2 S-S-S <sub>1</sub> -series	♂	4	7	18	6	4	9	68	115	
	♀	20	11	8	4	4	—	47		
3 S <sub>0</sub> S and substant	♂	36	8	18	9	0	3	83	166	
	♀	34	22	10	6	5	—	78		
4 S <sub>1</sub> and substant	♂	13	3	9	2	2	3	31	50	
	♀	9	3	3	4	2	—	21		

Ath = clinical atherosclerosis. N = ordinary diet. FR = diet restricted in saturated fat. PU = FR-diet and poly unsaturated fat added. S<sub>0</sub>, S<sub>1</sub>, S<sub>2</sub> = serum before and 4 and 7 hours after the fat meal.

latter diet with poly unsaturated fats added

B Groups studied as such in which each subject is represented by a single fat tolerance test. These groups were subdivided into male and female controls, hypercholesterolaemic, hyperlipaemic and subjects with and without clinical manifestations of atherosclerosis. A discrete group comprised normoglycaemic and normochole-

sterolaemic males with clinical atherosclerosis. The various groups in this category were<sup>1</sup>

1 The S<sub>0</sub>-S<sub>4</sub> Series (210 subjects) in which one serum specimen taken immediately before (S<sub>0</sub>) and another 4 hours after the fat meal (S<sub>4</sub>) were analyzed (see Table 2)

<sup>1</sup> Clinical data and the results of the fat tolerance test for each patient will be supplied on request from any interested reader

Mr. J. H. Jones      Mr. J. H. Jones      Mr. J. H. Jones      Mr. J. H. Jones

Sex	Group	years	$K_0$	$\sigma$	$\sigma_0$	$R$	$\tau$	$\tau_0$	$\sigma_0$	$q$	$\sigma_0$	$R$
Male	Controls	42	30.9	23	31	260	1	50	114	2.01	0.10	0.25
			2.8	6	7.7	6		9	0.04	0.12	0.01	0.01
	Hypertensive without insulin	11	43.9	25.6	0.7	315	17	17	1.50	2.24	0.12	1.02
			3.6	11	6.8	18	2	2	1.11	0.32	0.02	0.19
	Hypertensive with insulin	29	68.5	32.5	+2.0	260	+24	+24	1.00	2.20	0.10	0.09
			3.6	15	3.3	18	6	6	0.09	0.22	0.01	0.11
	Hypertensive without insulin	14	44.3	25.3	+0.6	327	+20	+20	0.84	1.23	0.20	3.11
			3.7	24	6.1	31	7	7	0.72	0.78	0.04	0.18
	Hypertensive with insulin	8	23.1	23.3	-2.4	213	+16	+16	3.05	0.27	0.17	1.49
			3.6	25	6.8	31	9	9	0.76	1.21	0.01	0.22
	Normotensive with glycerol	19	23.4	24.4	+2.4	263	+23	+23	1.52	2.14	0.06	1.06
			3.8	4	1.9	6	7	7	0.12	0.27	0.01	0.21
Female	Controls	36	42.6	27.0	+1.4	276	+20	+20	1.00	1.57	0.04	0.42
			2.4	8	2.5	7	2	2	0.04	0.14	0.01	0.01
	Hypertensive without insulin	48	42.7	30.3	+0.6	249	+24	+24	1.41	2.23	0.06	0.62
			2.2	26	4.1	16	11	11	0.11	0.21	0.02	0.29
	Hypertensive with insulin	18	23.9	21.8	+7.4	221	+26	+26	1.74	2.76	0.09	0.54
			2.7	11	2.7	16	2	2	0.10	0.21	0.01	0.22
	Hypertensive without insulin	8	42.8	24.7	+2.6	220	+27	+27	2.04	4.09	0.15	1.05
			3.8	23	6.6	24	3	3	0.12	0.77	0.02	0.20
	Hypertensive with insulin	8	23.0	47.0	+8.4	406	+24	+24	4.94	7.29	0.22	2.02
			2.3	12	8.3	22	9	9	1.06	2.21	0.11	0.21

$S_0$  and  $S_1$  = serum before and 4 hours after the fat load.  
 Figures in italics are standard error of the mean.

**Controls** This group comprised both healthy subjects and patients with orthopaedic disorders whose history and clinical picture exhibited no clinical manifestation of atherosclerosis, gallstone disease, hypertension<sup>1)</sup> or other non-orthopaedic disease. The clinical study included a general physical examination, Ecg, haemoglobin concentration, ESR and estimation of urinary sugar and albumin. Moreover, oscillography was done in every case and especial attention was paid to the presence of aortic atherosclerosis, xanthelasma and xanthoma. None of the controls had received any dietary supplement of polyunsaturated fats or otherwise altered his regular diet.

The majority of the controls were orthopaedic patients. Indeed 31 of the 42 male controls suffered from orthopaedic conditions, 10 of them from back pain of various types, predominantly sciatica. The corresponding figures for the female controls were 20 of 36 and 13 of 26. None of the controls had severe pains — practically all could walk to the lipid laboratory.

The ESR was between 10 and 20 mm in 11 and over 20 mm in 3 of the

78 controls. Among 3 men and one woman between 80 and 90 years of age, all of whom felt fit, took daily walks etc., one had oscillographic abnormalities and a PR interval of 0.80 seconds, while the Ecg revealed that another had auricular fibrillation, the third bundle-branch block and the fourth diphase T waves.

The controls were selected with a view to having all age decades between 20 and 90 years represented (See Table 3). Nevertheless, it turned out that the proportion of females up to 50 years of age was significantly higher than that of men ( $\chi^2 > 3.84$ ). No difference in lipids or turbidity could be demonstrated between these age groups for either males or females. Fig. 1 shows the glyceride-glycerol levels and turbidities of fasting serum, the respective means for men and women being 1.00 and 1.14 mMol/l and 0.10 and 0.075 turbidity units (cf. Tables 2 and 10).

The mean cholesterol and phospholipid levels of fasting serum were somewhat high for both male (255 and 270 mg%) and female (250 and 255 mg%) controls. When these groups were classified according to whether or not consanguineous relatives had gallstone disease, coronary affection or diabetes

<sup>1)</sup> except 3 cases: 38 years male 160/103, 65 years male 20/100 and 72 years female 180/103.

TABLE 3. Age distribution of control

	20-30	31-40	41-50	51-60	61-70	70 years	Total
Men	7	6	7	10	6	8	44
Women	6	8	12	7	1		34
Total	13	14	19	17	7	8	78

mellitus (ie a positive or negative family history) the mean lipid levels and turbidities set out in Table 4 were obtained. Evidently these values did not differ significantly for the groups with positive and negative family histories. The cholesterol level of fasting serum exceeded 300 mg in 12 of the female controls, 10 of whom were  $\geq 43$  years of age, i.e. during and after the menopause.

The association, if any between acataxia and serum lipids was studied by comparing 18 subjects (8 men and 9 women) classified as healthy controls and 40 patients (11 men and 9 women) with a diagnosis of acataxia, all being below 60 years of age. While the mean serum cholesterol levels for these two groups —  $256 \pm 0$  mg and  $277 \pm 13$  mg respectively — were not significantly unequal the corresponding glyceride-glycerol levels —  $0.90 \pm 0.08$  mMol/l and  $1.32 \pm 0.11$  mMol/l — did differ significantly.

95% range calculated on log glyceride-glycerol 0.4–1.1 mMol/l.

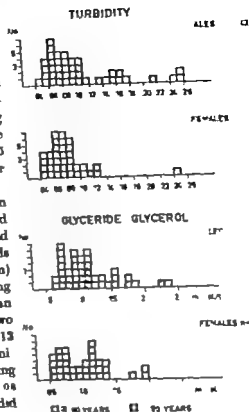


Fig 1 Frequency distribution of turbidity and glyceride-glycerol in serum of fasting controls

TABLE 4 Lipids and turbidity: fasting serum from controls classified according to family history of gallstone disease, coronary disease or diabetes mellitus

	History		Cholesterol mg <sup>100</sup>	Phospholipids mg <sup>100</sup>	Glyceride- glycerol mMol/l	Turbidity
M	negative	32	$248 \pm 7$	$249 \pm 7$	$1.14 \pm 0.17$	$0.10 \pm 0.01$
	positive	16	$276 \pm 11$	$255 \pm 8$	$1.13 \pm 0.12$	$0.10 \pm 0.02$
F women	negative	23	$278 \pm 18$	$273 \pm 10$	$1.03 \pm 0.07$	$0.09 \pm 0.04$
	positive	13	$281 \pm 12$	$279 \pm 10$	$0.90 \pm 0.10$	$0.09 \pm 0.01$

N: significant lipid differences could be demonstrated in fasting serum between male and female controls with and without positive family history.



TABLE 5 *Family and clinical histories of hypercholesterolaemics hyperlipaemics and normocholesterolaemics normoglycaemic atherosclerosis*

History	Hypercholesterolaemics				Hyperlipaemics				Normo- chol. <sup>1)</sup>	
	0 ath		+ th		0 ath		+ ath		+ th	
	n =	11 ♂	20 ♀	48 ♂	18 ♀	14 ♂	6 ♀	8 ♂	8 ♀	13 ♂
<i>Heredity</i>										
Hyperlipaemia and/or hypercholesterolaemia		4/4	16/15	2/	4/5	2/2	1/3	0/1	3/3	0/0
Myocardial infarction		6	15	10	11	1		4	3	4
Diabetes mellitus		1	8	0	1	3	1	1		3
<i>Clinical course</i>										
Xanthoma		5	10	0	3	2	0	1		17
Xanthelasm		1	8	4	4	0	0	5	4	
Arcus corneae		4	11	10	5	3	1	3		3
Gallstone disease		0	6	4	5	1	1	1	3	1
Hypertension		5	5	14	11	5	3	5	4	5
Fog during and after exercise		7	13	8	5	7		4	0	3
Angiography		1	1	10	9	3		0		0

<sup>1)</sup> Normocholesterolaemic normoglycaemic.

<sup>2)</sup> no positive/no. examined

*Clinical groups* Hypercholesterolaemics hyperlipaemics and atherosclerotics not belonging to either of these groups were subjected to fat tolerance tests. Patients with an active disease not associated with atherosclerosis were not included. Thus patients with active gallstone disease gastroduodenal ulceration and other gastrointestinal disorders were eliminated. So were patients with a known infection such as the "common cold" pneumonia or rheumatic disorders and convalescent surgical patients. Great care was taken not to include patients whose diet had been changed e.g. for voluntary weight reduction by fat restriction and/or addition of polyunsaturated fats.

Frequencies of clinical data of significance for blood lipid changes are given in Table 5 which provides information on heredity blood pressure gallstone disease occurrence of arcus corneae xanthoma and xanthelasma.

*Hypercholesterolaemics* The principal for selection of hypercholesterolaemics without and with atherosclerosis differed. The former group was selected because xanthelasma xanthoma or arcus corneae had called for analysis of serum lipids. Moreover the family history was penetrated in cases where some lipid abnormality had been demonstrated and if possible the relatives were examined. The group of hyper

cholesterolaemia with atherosclerosis was selected because the atherosclerotic disease had necessitated serum lipid analysis. Clinical particulars for the two groups are presented in Table 5. The table reveals that the incidence of hypertension was significantly higher in the atherosclerotic than in the non-atherosclerotic group, a difference associated with the inclusion of many patients with stenosis of the renal artery. The frequencies of other clinical factors exhibited no major differences. Abnormal glucose tolerances were equally common in the two groups.

The cholesterol and phospholipid levels of fasting serum tended to be higher in the non-atherosclerotic groups than in the atherosclerotic ones. This difference was significant when men and women were consolidated into a single group and was most likely due to the principles of selection. The converse situation applied to glyceride-glycerol that is the combined groups of atherosclerotics showed the higher serum glyceride-glycerol level.

The atherosclerotic and non-atherosclerotic groups of hypercholesterolaemias differed in yet another way, namely with respect to the higher mean age of the former (significantly for men plus women and for women alone). Accordingly age ( $\pm 1$  year) and sex matched groups were studied. These two groups (8 men and 11 women in each) exhibited no differences in the cholesterol, phospholipid and glyceride-glycerol levels of fasting serum (or serum 4 hours after a fat meal).

*Hyperlipaemia.* For the past several

years estimations of cholesterol in fasting serum have systematically been combined with serum turbidity measurements even mild turbidity increases ( $>0.10$ ) leading to estimation of the glycerides. The selection of hyperlipaemias with and without clinical atherosclerosis was based on criteria similar to those applying to the corresponding groups of hypercholesterolaemias. The frequencies of the clinical factors presented in Table 5 were similar in the hyperlipaemics with and without clinical atherosclerosis.

The four women with xanthelasma — reportedly a rare phenomenon in hyperlipaemia — all had serum glyceride-glycerol levels between and 3 mMol/l. The hyperlipaemic groups showed a significantly lower frequency of xanthelasma than the hypercholesterolaemic groups. These categories did not deviate in regard to the other clinical factors included in Table 5.

*Normoglyceridaemia with normocholesterolaemia with atherosclerosis.* To this group were assigned those patients who could not be included among either the hypercholesterolaemias or the hyperlipaemics, although in most cases numerous, often serial analyses of lipids in fasting serum had been carried out. This group exhibited no characteristic deviations from the hypercholesterolaemic and hyperlipaemic groups with respect to the clinical factors included in Table 5. The mean glyceride-glycerol level of fasting serum in this group significantly exceeded that for the controls (1.23 and 1.14 mMol/l respectively).

2. The 9 ~S ~S Series (115 sub-

TABLE 6. Fat tolerance tests the  $S - S_0 - S_1$  Series

Sex	Group	n	Age years	Cholesterol mg per cent		Phospholipids mg per cent		Glyceride-glycerol mMol/l				Turbidity	
				$S_0$	$S$	$S_0$	$S$	$S_0$	$S$	$S_1$	$S_2$	$S$	$S_2$
Male	Controls	4	46.3 31	356 3	270 8	331 9	30 8	75 8	118 0.10	4 0.19	4 0.14	0.67 0.01	0.51 0.05
	Hypercholesterolaemias without clinical atherosclerosis	7	40.1 34	363 38	363 36	31 37	308 3	326 18	161 0.13	3.08 0.39	2.39 0.62	0.13 0.03	1.03 0.12
	Hypercholesterolaemias with clinical atherosclerosis	18	50.8 5	329 11	331 1	37 14	303 7	320 8	18 0.14	3.38 0.90	2.69 0.38	0.09 0.07	0.40 0.10
	Hypolipidaemias without clinical atherosclerosis	6	47.8 2.9	400 27	416 24	409 0	345 22	331 22	377 1.10	4.00 1.39	5.68 1.66	0.33 0.04	1.39 0.72
	Hypolipidaemias with clinical atherosclerosis	4	62.5 2.9	378 63	374 68	373 63	347 50	340 83	361 0.63	2.89 1.10	5.51 1.3	0.23 0.09	0.90 0.15
	Normocholesterolaemia normo- glyceridaemia atherosclerosis	0	64 3.6	262 5	270 9	264 10	30 8	236 12	376 0.18	1.30 0.18	1.81 0.28	0.08 0.01	0.31 0.05
Female	Controls	40	41.6 31	83 13	283 14	271 12	278 12	303 12	103 0.10	1.75 0.15	1.37 0.13	0.06 0.01	0.44 0.03
	Hypercholesterolaemias without clinical atherosclerosis	11	47.1 3.8	394 22	412 1	393 10	369 16	400 17	148 0.11	2.28 0.21	1.67 0.15	0.08 0.03	0.34 0.05
	Hypercholesterolaemias with clinical atherosclerosis	8	61.3 2.3	340 1	340 14	344 11	323 14	322 9	14 0.1	2.66 0.32	2.77 0.28	0.43 0.01	0.51 0.12
	Hypolipidaemias without clinical atherosclerosis	4	61.6 9	364 56	363 37	357 51	360 48	362 45	308 0.56	4.36 1.11	4.18 0.75	0.17 0.02	0.67 0.10
	Hypolipidaemias with clinical atherosclerosis	4	60 1.8	403 67	417 73	436 61	408 37	506 36	532 1.03	8.33 1.43	10.87 0.77	0.18 0.01	1.76 0.05
	Controls	40	41.6 31	83 13	283 14	271 12	278 12	303 12	103 0.10	1.75 0.15	1.37 0.13	0.06 0.01	0.44 0.03
	Hypercholesterolaemias without clinical atherosclerosis	11	47.1 3.8	394 22	412 1	393 10	369 16	400 17	148 0.11	2.28 0.21	1.67 0.15	0.08 0.03	0.34 0.05

$S_0$ ,  $S$  and  $S_1$  = serum before 4 and 7 hours after the fat meal.

Figures in italics are standard errors of the mean.

) 1.8 turbidity value is lacking  $n=5$  yields the following turbidity values  $0.3 \pm 0.05$   $0.72 \pm 0.06$  and  $1.10 \pm 0.28$

jects) in which the cholesterol phospholipid and glyceride-glycerol levels and turbidity of serum were determined immediately before as well as 4 and 7 hours after the fat meal. (See Table 6.) With few exceptions the fasting and 4-hour values of these patients are included in the  $S-S_4$  Series. The group classification and results of serum lipid and turbidity estimations are shown in Table 6. Grouping was done according to the same principles as before. The controls were so chosen with respect to age as to make the decades from 20 to 60 years more or less equally numerous. Six men and women were over 60 years of age. The cholesterol level of fasting serum exceeded 300 mg % in 3 male and in no less than 8 female controls.

3 Serum and its supernatant  $S_0$  and  $S_1$  (supernatant after centrifugation at 25,000 g for 1 hour) were analyzed for glyceride-glycerol content and turbidity in 168 cases. These subjects have been classified by groups in Table 7.

4 Serum and its supernatant,  $S_7$ , where serum 7 hours after the fat meal and its supernatant were analyzed in 3 cases as under 3 (See Table 7).

#### Comment

It should be borne in mind that the control groups were not composed wholly of normal subjects. Two subgroups can be distinguished namely "healthy subject" and patients with somatic. These subgroups exhibited significantly unequal serum glyceride-glycerol levels, a fact worth

drawing attention to because initially sceleration was accepted as irrelevant in regard to serum lipids. CARLSON (1960) similarly accepted cervical spondylitis varicose veins, haemorrhoids and inguinal hernia. The mean serum glyceride-glycerol level for his normal men of all age groups (age range 26-73 years) was 1.19 mEq/l a value practically identical to the writer's 1.14 mEq/l for all the 4 male controls. The corresponding cholesterol and phospholipid levels of fasting serum — 48 and 85 mg % versus 30 and 254 mg % respectively — also agreed closely. Notably however CARLSON's and the writer's control groups had significantly dissimilar age distributions, the latter showing a predominance of the higher age groups (over 45 years) so that the mean age of all the male controls was 50.9 years as against 44.0 years for CARLSON's normal men. But there was no significant difference between the present controls over and under 50 years of age in either the cholesterol phospholipid and glyceride-glycerol levels or the turbidity of fasting serum.

The similarity with respect to all lipid factors between the writer's and CARLSON's control groups is worth stressing because STANBORG & SVENNERHOLM (1961) as well as CRAMER (1962) reported lower cholesterol and phospholipid levels and CRAMER had a lower glyceride-glycerol level as well. Conversely MALMCRONA (1960) obtained cholesterol values of more nearly the same order as the writer's. Though genetic and dietetic differences between the populations on the West and East coasts of Sweden (STANBORG

Group	Sex	Glyceride-glycerol mMol/l			Turbidity		
		I	II	I-II	I	II	I-II
0 hour							
Controls	♂	26	1.14 ± 0.08	1.07 ± 0.07	0.08 ± 0.03	0.09 ± 0.01	0.06 ± 0.00
	♀	34	1.0* ± 0.06	0.96 ± 0.06	0.03 ± 0.01	0.07 ± 0.01	0.03 ± 0.00
Hypercholesterolemia without clinical atherosclerosis	♂	8	1.40 ± 0.13	1.31 ± 0.11	-0.03 ± 0.04	0.13 ± 0.05	0.04 ± 0.02
	♀	23	1.35 ± 0.09	1.33 ± 0.08	0.0 ± 0.0*	0.09 ± 0.01	0.06 ± 0.00
Hypercholesterolemia with clinical atherosclerosis	♂	11	1.71 ± 0.14	1.66 ± 0.13	0.03 ± 0.03	0.09 ± 0.01	0.07 ± 0.00
	♀	10	1.81 ± 0.2	1.79 ± 0.1	0.03 ± 0.04	0.09 ± 0.01	0.06 ± 0.00
Hypertension without clinical atherosclerosis	♂	0	4.18 ± 0.67	3.41 ± 0.44	0.78 ± 0.39	0.35 ± 0.09	0.13 ± 0.0
	♀	6	3.87 ± 0.43	3.1 ± 0.43	0.07 ± 0.26	0.15 ± 0.0*	0.10 ± 0.01
Hypertension with clinical atherosclerosis	♂	0	3.96 ± 0.68	3.15 ± 0.30	0.81 ± 0.66	0.17 ± 0.01	0.11 ± 0.01
	♀	6	4.18 ± 1.3	3.64 ± 0.93	0.34 ± 0.41	0.23 ± 0.09	0.11 ± 0.01
Normocholesterolemia normotensive atherosclerosis	♂	9	1.61 ± 0.13	1.67 ± 0.17	-0.07 ± 0.02	0.09 ± 0.04	0.06 ± 0.00
4 hours							
Controls	♂	36	1.03 ± 0.14	1.48 ± 0.09	0.37 ± 0.08	0.3* ± 0.04	0.11 ± 0.01
	♀	34	1.60 ± 0.10	1.34 ± 0.09	0.27 ± 0.03	0.41 ± 0.03	0.11 ± 0.01
Hypercholesterolemia without clinical atherosclerosis	♂	8	2.43 ± 0.20	1.89 ± 0.21	0.56 ± 0.10	0.84 ± 0.15	0.13 ± 0.03
	♀	23	2.16 ± 0.14	1.9 ± 0.01	0.29 ± 0.06	0.43 ± 0.10	0.14 ± 0.0*
Hypercholesterolemia with clinical atherosclerosis	♂	18	2.01 ± 0.3	3.1 ± 0.14	0.70 ± 0.14	0.89 ± 0.11	0.13 ± 0.01
	♀	10	2.38 ± 0.22	2.99 ± 0.26	0.30 ± 0.11	0.48 ± 0.07	0.11 ± 0.01
Hypertension without clinical atherosclerosis	♂	0	6.81 ± 0.61	4.11 ± 0.27	0 ± 0.46	2.47 ± 0.51	0.20 ± 0.0*
	♀	6	4.17 ± 0.9	3.74 ± 0.74	0.43 ± 0.7	1.03 ± 0.30	0.19 ± 0.04
Hypertension with clinical atherosclerosis	♂	0	6.1 ± 1.04	3.73 ± 0.40	2.37 ± 0.3	1.23 ± 0.49	0.4 ± 0.05
	♀	6	3.73 ± 0.67	4.90 ± 0.57	1.32 ± 0.23	1.67 ± 0.4	0.17 ± 0.0*
Normocholesterolemia normotensive atherosclerosis	♂	8	3.75 ± 0.43	4.49 ± 0.34	1.22 ± 0.18	1.1 ± 0.37	0.13 ± 0.0*
8 hours							
Controls	♂	1	1.40 ± 0.24	1.31 ± 0.22	0.19 ± 0.06	0.50 ± 0.04	0.07 ± 0.01
	♀	9	1.11 ± 0.17	0.94 ± 0.14	0.1 ± 0.06	0.4 ± 0.06	0.07 ± 0.01
Hypercholesterolemia without clinical atherosclerosis	♂	1	1.40 ± 0.24	1.31 ± 0.22	0.19 ± 0.06	0.50 ± 0.04	0.07 ± 0.01
	♀	9	1.11 ± 0.17	0.94 ± 0.14	0.1 ± 0.06	0.4 ± 0.06	0.07 ± 0.01

& SVENSSON, 1961) have been proposed as factors responsible for these discrepancies, they might also be due simply to different criteria for the selection of subjects. The writer's observations seem to support the latter view.

The groups of hypercholesterolaemia, hyperlipaemia and normoglycaemic normocholesterolaemia with atherosclerosis are rather diffusely demarcated. The fact that classification into these groups often was based on serial estimations explains why the hyperlipaemia included patients with substantially normal lipid levels of fasting serum on the day of the fat tolerance test. With the present classification some patients would perhaps be included among the hyperlipaemia because their serum lipids for some months exhibited a hyperlipaemic pat-

tern, whereupon several years with entirely normal serum lipids might elapse before another such period begins (of GROOVER, 1960).

Nor are the groups with and without atherosclerosis distinctly demarcated except that the latter had a significantly lower mean age. The frequency of such procedures as say angiography must obviously be low in the symptomless group and high in the group with atherosclerotic manifestations. The difficulty of classifying cases is illustrated by the following example. A man of 28 without cardiac distress and a normal Ecg during exercise was subjected to coronary angiography owing to several cases of coronary disease in the family. The angiograms disclosed total occlusion of the right coronary artery and atherosclerotic like changes in the left coronary artery.

Group	Box n	Glyceride-glycerol mM l/l			Turbid ty		
		I	II	I-II	I	II	I-II
0 hours							
Controls	♂ 26	1.14±0.08	1.07±0.07	0.08±0.03	0.09±0.01	0.06±0.00	0.03±0.01
	♀ 34	1.02±0.06	0.99±0.06	0.03±0.01	0.07±0.01	0.03±0.00	0.02±0.00
Hypercholesterolaemia without clinicaltherosclerosis	♂ 8	1.49±0.16	1.51±0.16	-0.03±0.04	0.13±0.03	0.09±0.04	0.04±0.03
	♀ 23	1.35±0.09	1.33±0.08	0.0±0.03	0.09±0.01	0.06±0.00	0.03±0.01
Hypercholesterolaemia with clinical atherosclerosis	♂ 18	1.1±0.14	1.06±0.13	0.03±0.03	0.09±0.01	0.07±0.00	0.02±0.00
	♀ 10	1.81±0.22	1.79±0.1	0.03±0.04	0.09±0.01	0.06±0.00	0.03±0.01
Hypolipidaemia without clinicaltherosclerosis	♂ 9	4.18±0.67	3.31±0.44	0.78±0.39	0.33±0.09	0.13±0.03	0.20±0.08
	♀ 6	9±0.45	2.91±0.43	0.07±0.26	0.15±0.03	0.10±0.01	0.03±0.03
Hypolipidaemia with clinical atherosclerosis	♂ 9	3.96±0.68	3.15±0.30	0.81±0.66	0.17±0.01	0.11±0.01	0.06±0.01
	♀ 6	4.18±1.33	3.64±0.93	0.54±0.41	0.23±0.09	0.11±0.01	0.12±0.06
Normocholesterolaemia normo glyceridaemiatherosclerosis	♂ 8	1.81±0.15	1.67±0.17	-0.0±0.0*	0.06±0.04	0.06±0.00	0.0±0.00
4 hours							
Control	♂ 26	0.9±0.14	1.46±0.09	0.57±0.08	0.6±0.04	0.11±0.01	0.41±0.04
	♀ 34	1.60±0.10	1.34±0.09	0.27±0.03	0.41±0.03	0.11±0.01	0.30±0.03
Hypercholesterolaemia without clinicaltherosclerosis	♂ 8	2.43±0.20	1.69±0.21	0.76±0.10	0.64±0.15	0.13±0.03	0.1±0.15
	♀ 23	1.18±0.14	1.9±0.01	0.39±0.06	0.53±0.10	0.14±0.03	0.39±0.06
Hypercholesterolaemia with clinicaltherosclerosis	♂ 18	3.01±0.25	2.31±0.14	0.70±0.14	0.69±0.11	0.13±0.01	0.57±0.11
	♀ 10	5.68±0.22	2.59±0.16	0.30±0.11	0.49±0.07	0.11±0.01	0.38±0.07
Hypolipidaemia without clinicaltherosclerosis	♂ 9	6.81±0.61	4.11±0.27	~0±0.46	4.7±0.31	0.20±0.0*	~0.7±0.50
	♀ 6	4.17±0.79	3.74±0.74	0.43±0.5*	1.03±0.30	0.19±0.04	0.85±0.18
Hypolipidaemia with clinicaltherosclerosis	♂ 9	6.13±1.04	2.73±0.40	3.7±0.3	1.33±0.20	0.34±0.03	1.09±0.27
	♀ 6	3.73±0.67	4.50±0.5*	1.3±0.23	1.67±0.4	0.17±0.03	1.50±0.40
Normocholesterolaemia normo glyceridaemiatherosclerosis	♂ 8	2.72±0.4	2.49±0.34	1.4*±0.16	1.21±0.37	0.13±0.0	1.06±0.35
7 hours							
Controls	♂ 1	1.30±0.4	1.31±0.23	0.19±0.06	0.20±0.04	0.07±0.01	0.13±0.03
	♀ 9	1.11±0.17	0.94±0.14	0.1±0.06	0.22±0.03	0.0*±0.004	0.15±0.02
Hypercholesterolaemia without clinicaltherosclerosis with							

*Glyceride-glycerol determination* The method described by CARLSON & WADSTRÖM (1959) was used in the early stages of the present investigation and CARLSON's (1959) modification thereof in its later stages.

For every series of glyceride-glycerol determinations, a special conversion factor was computed from the results on at least 4 standard tripalmitin references and 4 blanks.

Every cholesterol phospholipid and glyceride-glycerol determination was duplicated on the extract and every series of determinations included standard references. Analytical errors of the methods are presented in Table 2.

*The fat tolerance test* Before breakfast after 1 hours fasting, the patient was given a standard meal of 200 ml of cream (40% butter fat). Blood samples for estimation of lipids were drawn immediately before and 4 hours after — in a minority of patients also 7 hours after — ingestion of the cream. In the meantime the in-patient had the freedom of the ward. The out patient was requested to keep reasonably still in the morning before the fat tolerance

test and while it was in progress. During this period the patients were not allowed to eat and should refrain from smoking and drinking.

The blood samples were at first drawn into oxalated tubes (3 mg sodium oxalate per ml.) But as pointed out by SEGERSTEDT & SCHÖNBERGER (1935) volume for volume the lipid levels of oxalated plasma are some 15 per cent lower than those of serum or heparinized plasma as a consequence of altered plasma volume. Against this background the following check was made. The differences between serum and plasma in turbidity and cholesterol phospholipid and glyceride-glycerol contents were determined in 15 fasting blood samples and 15 four hours after a standard fat meal. The cholesterol, phospholipid and glyceride-glycerol levels of serum turned out to be on average 0.7 per cent (range: 0—+29%) higher than the corresponding plasma levels, regardless of whether the samples were fasting or postprandial. The mean lipid levels of serum and their ranges were: 277 mg % (162—400 mg %) cho-

TABLE 2. Analytical error of the methods

		Number of duplicates	Range	Mean	Analytical error in per cent
Cholesterol mg %	serum <sup>1)</sup>	50	149—409	304	1.81
	extract	100	214—873	311	0.85
Phospholipids mg %	serum <sup>1)</sup>	30	178—443	283	2.44
	extract	100	183—453	293	2.16
Glyceride-glycerol mMol/l	serum <sup>1)</sup>	100	0.43—8.56	1.34	2.72
	extract	100	0.43—8.56	1.34	2.15

<sup>1)</sup> single determinations on duplicate extracts of serum.

<sup>2)</sup> duplicate determinations on duplicate extracts of serum.



## METHODS

*Turbidity determination* The turbidities of serum and plasma were determined as optical density at 650  $m\mu$  10 mm pathway and room temperature in a Beckman B Spectrophotometer using a distilled water blank. The wavelength 650  $m\mu$  was used for the turbidity measurements in the present investigation because it has been adopted by the majority of previous authors (BLOCK, BARKER & MAX 1951; HERZSTEIN 1953; WOLDOW, CHAPMAN & EVANS 1954; BRONTE-STEWART & BLACKBURN 1958; KUO & JOYNER 1955 etc.) A serum sample whose turbidity exceeded 1.50 units was diluted with 4 volumes of physiological saline 1.50 turbidity units being the upper limit beyond which the dilution curve for chylomicrons ceases to be linear or nearly so.

*Extraction of lipids* (after SVANBORG & SVENNERHOLM 1958) 1 ml serum was pipetted into a 25 ml Erlenmeyer flask containing 15 ml chloroform-methanol (1:1 by volume). After shaking the mixture was filtered into a graduated cylinder through a paper boiled in alcohol. The flask was rinsed twice with 3 ml chloroform-methanol and the filter paper cone with 3 ml chloroform-methanol and then once with 3 ml pure chloroform. To remove contaminants phase partition was achieved by adding 3–5 ml physio-

logical saline shaking the cylinder violently for 1 minute and then letting it stand for 24 hours. The upper layer was discarded and the lower layer as well as the cylinder walls were washed twice with 3 ml of cleaning fluid (the upper phase formed after mixing 150 ml chloroform, 120 ml methanol and 50 ml physiological saline). After discarding the upper layer pure chloroform was added to the lower up to the 25 ml mark. The resultant extract was used for lipid determination.

*Cholesterol determination* Cholesterol was determined by a modification of the method given by THEORELL (1920, 1931). This method has been submitted to extensive comparison with the SPERRI & WEBB (1950) procedure. At all serum levels the present method gave 4.6–4.9 per cent higher values (HOOD & ANGERVALL, 1957, 1959; CRAMÉR & ISAKSSON 1959). In 50 duplicate determinations there was no significant difference between the mean cholesterol content of the chloroform-methanol extract and that of the acetone-ethanol extract. Whenever both extractants were used in the present investigation the two cholesterol values were averaged.

*Phospholipid determination* Serum phospholipids were estimated according to SVANBORG & SVENNERHOLM (1961).

**Glyceride-glycerol determination.** The method described by CARLSON & WANDERMAN (1936) was used in the early stages of the present investigation and CARLSON's (1938) modification thereof in its later stages.

For every series of glyceride-glycerol determinations, a special conversion factor was computed from the results on at least 4 standard tripalmitin references and 4 blanks.

Every cholesterol phospholipid and glyceride-glycerol determination was duplicated on the extract and every series of determinations included stand and references. Analytical errors of the methods are presented in Table 3.

**The fat tolerance test.** Before breakfast, after 1 hour's fasting the patient was given a standard meal of 500 ml of cream (40% butter fat). Blood samples for estimation of lipids were drawn immediately before and 4 hours after — in a minority of patients also 7 hours after — ingestion of the cream. In the meantime the inpatient had the freedom of the ward. The out patient was requested to keep reasonably still in the morning before the fat tolerance

test and while it was in progress. During this period the patients were not allowed to eat and should refrain from smoking and drinking.

The blood samples were at first drawn into oxalated tubes (1 mg sodium oxalate per ml.) But as pointed out by GREEN & SCHOKENBACH (1935) volume for volume the lipid levels of oxalated plasma are some 15 per cent lower than those of serum or heparinized plasma as a consequence of altered plasma volume. Against this background, the following check was made. The differences between serum and plasma in turbidity and cholesterol phospholipid and glyceride-glycerol contents were determined in 15 fasting blood samples and 16 four hours after a standard fat meal. The cholesterol, phospholipid and glyceride-glycerol levels of serum turned out to be on average 9.7 per cent (range 0—+20%) higher than the corresponding plasma levels, regardless of whether the samples were fasting or postprandial. The mean lipid levels of serum and their ranges were 477 mg% (16—602 mg%) cho-

TABLE 3. Analytical error of the methods

		Number of Duplications	Range	Mean	Analytical error in per cent
Cholesterol mg%	serum <sup>1</sup>	40	140—608	306	1.61
	extract	100	214—573	311	0.86
Phospholipids mg%	serum <sup>1</sup>	80	373—583	468	2.44
	extract	100	183—533	300	2.19
Glyceride glycerol mEq/100 ml	serum <sup>1</sup>	100	11.23—39.50	1.86	3.72
	extract	100	8.63—38.86	1.95	3.16

<sup>1</sup> single determinations on duplicate extracts of serum.

<sup>2</sup> duplicate determinations on duplicate extracts of serum.

lesterol 285 mg% (174—413 mg%) phospholipids and 1.88 mMol/l (0.52—5.02 mMol/l) glyceride-glycerol. In order to render them comparable with the corresponding serum level plasma values for cholesterol phospholipids and glyceride-glycerol obtained in the first part of the series were adjusted to 110 per cent of the value actually estimated.

The turbidity estimations showed a much greater range of variation. Thus the serum turbidity averaged 0.20 units and ranged from 0.05 to 1.33 units. The serum turbidity was on average 0.3 per cent higher than the corresponding plasma turbidity (range —34%—+44%). On inspection oxalated plasma samples are occasionally found to contain minute particles which can be made to precipitate by centrifugation at high speeds. Calcium oxalate might well contribute to the turbidity of oxalated plasma. No correction was applied to the turbidity values of plasma.

Following centrifugation of each blood sample at 1000 *g* for 20 minutes the serum supernatant was pipetted

off and its turbidity and cholesterol phospholipid and glyceride-glycerol levels determined. These serum samples from a proportion of the patients were thereupon recentrifuged at 25 000 *g* for 60 minutes in a Phyvo-Pirouette water cooled High Speed Centrifuge Type 804. The serum supernatant thus obtained was aspirated and subjected to similar determinations.

In 10 cases the values of the lipid factors 4 hours after ingestion of the fat meal were estimated in two separate blood samples. Thus after centrifugation of each such sample at 1000 *g* for 20 minutes on two occasions, the cholesterol phospholipid and glyceride-glycerol levels and turbidity of its serum supernatant were determined, whereupon each such serum portion was centrifuged at 25 000 *g* for 60 minutes on two occasions and the corresponding lipid factors were estimated in its serum supernatant. The results of these analyses are presented in Table 9. Except for turbidity the errors of serum centrifugation at 25 000 *g* were smaller than at 1000 *g*. Hence the chylomicron phase is well defined.

TABLE 9. Errors / serum centrifugation at 1000 *g* and 25 000 *g*

	Centrifugal force <i>g</i>	Range	Mean	Anal.tical error
Cholesterol mg%	1 000	143—397	239	3.3
	25 000	141—390	229	6
Phospholipid mg%	1 000	164—303	262	4
	25 000	158—380	232	2
Glyceride glycerol mMol/l	1 000	0.98—4.59	1.07	4
	25 000	0.80—4.59	1.5	4.4
Turbidity	1 000	0.043—1.8	0.552	—
	25 000	0.043—0.70	0.089	2.5

## DUPLICATE FAT TOLERANCE TESTS

Duplicate fat tolerance tests, with determinations of cholesterol, phospholipids, glyceride-glycerol and turbidity in serum before and 4 hours after ingestion of the fat meal were performed on 57 subjects.

No systematic difference could be demonstrated between the 1st and 2nd fat tolerance tests. Neither the cholesterol, phospholipid and glyceride-glycerol levels nor the turbidity of serum differed significantly on the two occasions. Accordingly the two fat tolerance tests were assumed to be made under similar conditions.

Table 10 presents means ( $\pm$  standard errors) for the lipid levels and turbidity of serum on the two occasions and standard deviations in per cent of the difference in the respective lipid and turbidity between the two occasions. The table reveals that the standard deviation of the difference in fasting serum varied between 10 and 11 per cent of the mean for cholesterol, 12 and 13 per cent for phospholipids, 1 and 2 per cent for glyceride-glycerol and 64 and 271 per cent for turbidity. The great turbidity dispersion of fasting serum from hyperlipaemic was caused by a single excessive value of 3.8. If this value is disregarded, the spread will be 60.2 per cent of the group of hyperlipaemics and 8.2 per cent for the whole group.

The associations between the 4-hour values and the corresponding 0-hour values were studied by plotting the respective differences. The strength of the associations was then studied with the aid of  $\chi^2$  analysis (Cf footnote p 34).

Turbidity (No significant) association could be demonstrated within any of the groups between the differences in 4-hour and fasting values on the two

1) 5 per cent level.

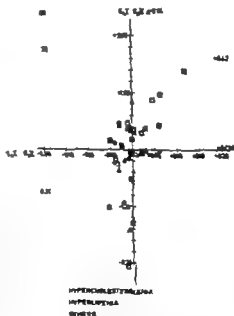


Fig. 2. Association between differences of turbidity in sera 4 hours after ( $S_1 - S_0 II$ ) and before ( $S_0 I - S_0 II$ ) duplicate standard fat meals.

lesterol 285 mg% (174—413 mg%) phospholipids and 1.88 mMol/l (0.52—5.9 mMol/l) glyceride-glycerol. In order to render them comparable with the corresponding serum level plasma values for cholesterol, phospholipids and glyceride-glycerol obtained in the first part of the series were adjusted to 110 per cent of the value actually estimated.

The turbidity estimations showed a much greater range of variation. Thus the serum turbidity averaged 0.30 units and ranged from 0.03 to 1.35 units. The serum turbidity was on average 0.6 per cent higher than the corresponding plasma turbidity (range —34%—+44%). On inspection oxalated plasma samples are occasionally found to contain minute particles which can be made to precipitate by centrifugation at high speeds. Calcium oxalate might well contribute to the turbidity of oxalated plasma. No correction was applied to the turbidity values of plasma.

Following centrifugation of each blood sample at 1000 g for 20 minutes the serum supernatant was pipetted

off and its turbidity and cholesterol, phospholipid and glyceride-glycerol levels determined. These serum samples from a proportion of the patients were thereupon recentrifuged at 25 000 g for 60 minutes in a Phyve-Pirouette water cooled High Speed Centrifuge Type 804. The serum supernatant thus obtained was aspirated and subjected to similar determinations.

In 10 cases the values of the lipid factors 4 hours after ingestion of the fat meal were estimated in two separate blood samples. Thus after centrifugation of each such sample at 1000 g for 20 minutes on two occasions, the cholesterol, phospholipid and glyceride-glycerol levels and turbidity of its serum supernatant were determined whereupon each such serum portion was centrifuged at 25 000 g for 60 minutes on two occasions and the corresponding lipid factors were estimated in its serum supernatant. The results of these analyses are presented in Table 9. Except for turbidity the errors of serum centrifugation at 25 000 g were smaller than at 1000 g. Hence the ohylomicron phase is well defined.

TABLE 9. Errors / serum centrifugation at 1000 g and 25 000 g

	Centrif. g force in g	Ra. g	Mean	Anal. total error in
Cholesterol mg%	1 000	140—307	229	2.3
	25 000	141—380	229	—6
Phospholipid mg%	1 000	164—393	262	4
	25 000	158—380	232	3
Glyceride-glycerol mM l/l	1 000	0.88—4.59	2.07	4.8
	25 000	0.80—2.09	1.53	4.4
Turbidity	1 000	0.063—1.87	0.55	—
	25 000	0.045—0.20	0.084	+0.3

occasions. A positive and significant association emerged when the hypercholesterolaemias and the others were combined into a single group or the entire series was studied (See fig  $\chi^2 > 3.84$ ). Thus, for a single individual the fasting and 4-hour turbidities of serum at two fat tolerance tests are associated in the sense that a higher fasting turbidity on the second occasion often is followed by a higher 4-hour value.

**Glyceride-Glycerol Differences in 4 hour and fasting values** between two fat tolerance tests are more strongly associated than those for turbidity the association being positive and significant for the hyperlipaemias and the hypercholesterolaemias as well as for the series as a whole (See fig 2). Table 11 presents the results of duplicate fat tolerance tests on 8 hyperlipaemias in whom the glyceride-glycerol difference in fasting serum exceeded 1 mUol/l. Notwithstanding the considerable spread of the fasting values the 4 hour value was so closely related to the 0-hour value that their ratios were closely similar in 7 of the 8 patients.

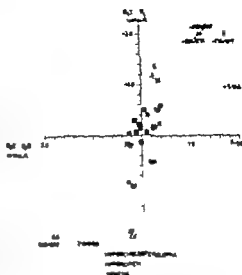


Fig 2 Association between differences of glyceride-glycerol in sera 4 hours after (S I - N II) and before (S<sub>0</sub> I - S<sub>0</sub> II) duplicate standard fat meals.

The mean ratio of the 4-hour and fasting serum glyceride-glycerol levels (Cf Chapter V II) was 1.79 for the 30 hypercholesterolaemias, 1.66 for the 1 hyperlipaemia and 1.74 for the 10 others the standard deviations of the difference between the two determinations being 0.37, 0.41 and 0.22.

TABLE 11 Glyceride-glycerol ratio (S<sub>4</sub>/S<sub>0</sub>) in hyperlipaemias whose S<sub>0</sub> value at duplicate tests exceeds 1 mUol/l

Individual and age	S <sub>0</sub> I	S <sub>0</sub> II	S <sub>4</sub> I	S <sub>4</sub> II	S <sub>4</sub> /S <sub>0</sub> I	S <sub>4</sub> /S <sub>0</sub> II
K.O. 23	9.88	18.71	9.82	18.83	1.01	1.01
L.A. 37	3.83	5.40	6.97	6.87	1.82	1.63
A.V. 43	97	278	1014	2.96	1.13	1.87
H.L. 19	4.82	8.13	12.83	8.90	1.46	1.37
V.N. 19	4.88	1.84	9.23	4.71	1.91	2.17
E.A. 74	10.87	8.81	8.88	7.90	0.82	1.43
H.L. 26	3.43	4.36	4.83	8.98	1.41	1.37
K.J. 64	2.41	4.80	3.28	6.83	1.25	1.31

TABLE 10 *Dupl cate fat tolerance test (FTT I and II)*

	Serum lipids before the fat meal				Serum lipids 4 hours after the fat meal			
	Cholesterol mg%	Phospholipids mg%	Glyceride- glycerol ml/ol/l	Turbidity	Cholesterol mg%	Phospholipids mg%	Glyceride- glycerol ml/l	Turbidity
<i>Hyperchol at</i>								
<i>Series n=30</i>								
FTT I	320 ± 11	79 ± 9	1.38 ± 0.10	0.088 ± 0.006	330 ± 13	323 ± 11	1.64 ± 0.13	0.88 ± 0.11
FTT II	329 ± 11	304 ± 9	1.43 ± 0.13	0.094 ± 0.01	332 ± 11	318 ± 10	2.5 ± 0.20	0.08 ± 0.04
diff I-II	35.6	36.1	0.340	0.071	31.4	34.3	0.773	0.789
diff in %								
f FTT mean	10.8	1--	1.3	80.2	9.3	10.6	0.3	84.9
<i>Hyperchol po n=17</i>								
FTT I	342 ± 23	328 ± 1	4.64 ± 0.75	0.581 ± 0.037	343 ± 23	349 ± 19	6.63 ± 0.73	2.27 ± 0.36
FTT II	351 ± 23	318 ± 15	4.43 ± 0.94	0.436 ± 0.44	342 ± 23	349 ± 20	6.33 ± 0.23	1.80 ± 0.30
diff I-II	37.1	40--	3.348	0.689	40.4	63.2	2.726	1.103
diff in %	10.8	1.3	3.0	43.3	11.8	18.1	36.6	54.4
f FTT mean								
<i>Cholere n=10</i>								
FTT I	211 ± 10	224 ± 10	1.64 ± 0.16	0.083 ± 0.014	221 ± 11	249 ± 0	2.67 ± 0.31	0.83 ± 0.18
FTT II	210 ± 12	18 ± 14	1.46 ± 0.16	0.083 ± 0.006	223 ± 14	23 ± 16	2.59 ± 0.37	1.19 ± 0.37
diff I-II	1	44.3	0.33	0.061	2.3	55--	0.561	0.755
diff in %								
f FTT mean	10--	19.9	1.1	63.9	14.7	2	21.0	80.3
<i>Total n=57</i>								
FTT I	312 ± 11	403 ± 0	1.31 ± 0.29	0.147 ± 0.03	319 ± 11	318 ± 9	2.83 ± 0.34	0.28 ± 0.13
FTT II	216 ± 11	403 ± 8	2.33 ± 0.33	0.201 ± 0.03	216 ± 11	218 ± 10	3.85 ± 0.38	1.27 ± 0.14
diff I-II	33.8	39.0	1.014	0.473	24.0	47.7	2.077	0.874
diff in %								
f FTT mean	10.8	13.3	7.6	47.1	10.	13.0	34	84.0

TABLE 12. Serum cholesterol changes at duplicate fat tolerance tests

			Cholesterol mg per cent				
			$N_s$		Cholesterol change		Difference
			A lower	B higher	A	B	A - B
Hypercholesterolaemia	)	30	215.2 $\pm$ 10.8	242.8 $\pm$ 10.7	+ 8.0 $\pm$ 2.6 <sup>7)</sup>	+ 2.1 $\pm$ 2.0	+ 5.9 $\pm$ 4.7
		14	316.3 $\pm$ 20.0	361.8 $\pm$ 19.2	+ 18.0 $\pm$ 8.1 <sup>7)</sup>	- 1.4 $\pm$ 5.4	+ 17.4 $\pm$ 7.3 <sup>7)</sup>
Hyperlipaemia	)	17	331.4 $\pm$ 22.4	352.3 $\pm$ 24.5	+ 6.9 $\pm$ 3.9	- 15.4 $\pm$ 8.2	+ 22.3 $\pm$ 10.1 <sup>7)</sup>
		13	344.4 $\pm$ 26.8	332.1 $\pm$ 23.6	+ 4.2 $\pm$ 4.7	- 23.2 $\pm$ 8.8 <sup>7)</sup>	+ 27.4 $\pm$ 12.8 <sup>7)</sup>
Others	)	10	207.0 $\pm$ 10.0	222.9 $\pm$ 11.8	+ 7.1 $\pm$ 17.3	+ 6.1 $\pm$ 2.7 <sup>7)</sup>	+ 1.0 $\pm$ 8.5
		2	217.0 $\pm$ 9.0	260.8 $\pm$ 7.8	- 3.8 $\pm$ 4.5	+ 7.0 $\pm$ 3.0	- 10.8 $\pm$ 7.5
Total	)	57	301.0 $\pm$ 10.8	327.8 $\pm$ 11.4	+ 7.8 $\pm$ 2.4 <sup>7)</sup>	- 3.4 $\pm$ 2.1	+ 10.0 $\pm$ 4.1 <sup>7)</sup>
		29	321.0 $\pm$ 14.2	362.8 $\pm$ 14.3	+ 9.2 $\pm$ 2.0 <sup>7)</sup>	- 10.4 $\pm$ 3.1 <sup>7)</sup>	+ 19.6 $\pm$ 6.7 <sup>7)</sup>

) Subjects with fasting serum cholesterol difference exceeding 20 mg%.

7) = significant difference

between the groups with lower (A) and higher (B) fasting cholesterol levels was not significant. The glyceride-glycerol ratios in these two groups were not unequal (1.77 and 1.73 respectively).

**Phospholipids** When the phospholipid differences between fasting sera and between 4-hours sera for two fat tolerance tests are plotted it becomes apparent that these differences seem negatively correlated. (Fig. 5) This is also shown by Table 13 in which the lower phospholipid level of fasting serum is called A and the higher B. Table III discloses that the mean phospholipid change 4 hours after a fat meal was significantly greater for Group A than for Group B in the hypercholesterolaemias. The hyperlipaemics and others exhibited similar tendencies. In addition the difference between the phospholipid changes was

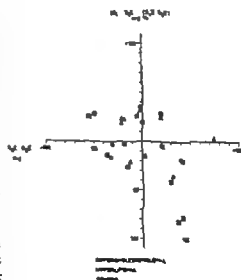


Fig. 5. Association between differences in serum phospholipid changes between 4 and 0 hours  $[(S_4 I - S_4 II) - (S_4 II - S_4 II)]$  and phospholipid differences of fasting sera  $(S_0 I - S_0 II)$  in duplicate standard fat meals.



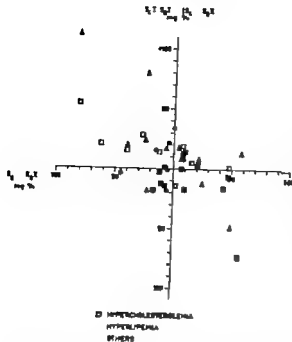


Fig 4 Association between differences in serum cholesterol changes between 4 and 0 hours  $[(S_4I - S_4II) - (S_0I - S_0II)]$  and cholesterol differences of fasting sera  $(S_0I - S_0II)$  at duplicate standard fat meals.

respectively. The corresponding figures for the series as a whole were 1.74 and 0.36. In other words if this ratio is regarded as a measure of the effect of the fat load its "error of method" would be 0.20 or around 20 per cent. The coefficients of correlation between the glyceride:glycerol ratios at the 1st and 2nd fat tolerance tests were 0.87 and 0.83 for respectively the hypercholesterolaemias and the hyperlipaemias.

**Cholesterol.** In the present context it is appropriate to call the difference between the cholesterol levels of fast ing serum and 4 hour serum the cholesterol change.

Differences between the two fast ing cholesterol levels as well as be

tween the two 4 hour cholesterol levels have been plotted in Fig 4 which discloses that the correlation between fasting and 4 hour values is negative.

When the pairs of fasting cholesterol values were arranged with the lower in one group (A) and the higher in another group (B) it appeared that the sera with the lower cholesterol values showed the greater mean cholesterol change 4 hours after the fat meal (Table 12). The difference in cholesterol change between Groups A and B is significant for the hyperlipaemias and for the hyperlipaemias plus hypercholesterolaemias and for the whole group ( $n = 57$ ).

When only patients with cholesterol differences between the fasting sera of more than 20 mg% were taken into consideration the result was as shown in Table 12. The cholesterol change in A now became significantly greater than that in B for the hyperlipaemias, the hypercholesterolaemias and the group as a whole. This suggests that a hypercholesterolaemic or hyperlipaemic patient's serum cholesterol change in response to fat loading depends mainly upon the cholesterol level of his fasting serum at the time of the fat meal. The cholesterol change will be largest if his fasting cholesterol level is near his own lower limit.

No significant association could be demonstrated between the cholesterol change and the glyceride:glycerol ratio ( $\chi^2 < 3.85$ ).

In all patient categories the mean glyceride:glycerol was lower in the group of fasting sera with the lower cholesterol level although the difference

TABLE 14

	Association between glyceride-glycerol and		
	Cholesterol	Phospholipids	Turbidity
Hypercholesterolaemia - 20	sign.	sign.	sign.
Hyperlipaemia - 17	—	sign.	sign.
*Others - 10	—	—	—

be accompanied by a lower glyceride-glycerol value

The significant associations between turbidity and glyceride-glycerol are surprising especially in the hypercholesterolaemic group with its small turbidity differences (mean 0.04). The correlation coefficients  $r$  for the hypercholesterolaemics, hyperlipaemics and others and for the series as a whole were 0.47, 0.97, 0.90 and 0.85 respectively. These findings illustrate the value of turbidity measurements as a test for neutral fat changes in the individual subjects.

In 4-hour sera the only association studied was that between turbidity and glyceride-glycerol and this association was not significant for any of the subgroups, nor for the series as a whole ( $n = 57$ ). The association between turbidity and glyceride-glycerol in the chylomicron phase was studied in 43 cases and proved to be significant for the complete series as well as for hypercholesterolaemics ( $n = 23$ ) and hyperlipaemics ( $n = 1$ ). The glyceride-glycerol content of the chylomicron phase and that of serum exhibited a significant positive correlation.

*Comment.* The present investigation demonstrated that the 4 hour and

fasting differences in serum turbidity and glyceride-glycerol level at duplicate fat tolerance tests are significantly correlated.

A number of authors have expressed the opinion that duplicate fat tolerance tests yield fairly similar results, most of them merely having compared the curves for serum turbidity or total serum lipids after repeated fat meals (MORETON 1950; MESSINGER & PORO SOWERS, 1953; BARNITT 1956; OSMON ZIEN & WHEATON 1957; BRONTE STEWARD & BLACKBURN 1958). The 4-hour and fasting turbidity and glyceride-glycerol differences observed in the present investigation varied considerably at duplicate fat tolerance tests, suggesting that analysis of association is a much better measure of variability than curve tracing.

The results thus imply that the higher the subject's fasting serum glyceride-glycerol level or turbidity, the higher his values 4 hours after a fat meal. This result could be due to more rapid fat absorption, but there exists no evidence indicating that the rate of fat absorption is accelerated when the serum neutral fat level is enhanced. The reason might be reduced fat removal from the blood stream.

TABLE 13 Serum phospholipid changes at duplicate fat tolerance tests

	Phospholipids in mg per cent				
	S		Phospholipid change		Difference A-B
	A lower	B higher	A	B	
Hypercholesterolaemia n=30	385.2 ± 9.0	315.6 ± 8.4	+3.0 ± 6.2 <sup>2)</sup>	+17.6 ± 3.8 <sup>2)</sup>	+14.1 ± 3.3 <sup>2)</sup>
Hyperlipaemia n=17	307.2 ± 14.8	337.9 ± 16.9	+24.1 ± 0.6 <sup>2)</sup>	+18.9 ± 31.6	+13.1 ± 13.6
Others n=10	303.8 ± 11.5	337.6 ± 10.0	+27.5 ± 7.7 <sup>2)</sup>	+18.6 ± 5.1	+10 ± 10.
Total	277.5 ± 8.2	308.6 ± 8.2	+32.2 ± 4.5 <sup>2)</sup>	+17.8 ± 3.4 <sup>2)</sup>	+14.3 ± 3.2 <sup>2)</sup>

<sup>2)</sup> = significant difference

also significant for the series as a whole. Nothing new emerged when the analysis was based on duplicate fat tolerance tests at which the phospholipid difference of fasting serum exceeded 20 mg%.

No significant association could be demonstrated within subjects between the serum phospholipid change and the serum glyceride-glycerol ratio.

Whereas the hypercholesterolaemics and the series as a whole exhibited a significantly higher mean glyceride-glycerol content of those sera that had the higher mean phospholipid level, the hyperlipaemics and others merely displayed similar tendencies. Groups A and B had similar mean glyceride-glycerol ratios.

*Association between the glyceride-glycerol level of fasting serum and its cholesterol phospholipids and turbidity.* In the individual subject the respective differences in fasting sera

were studied by  $\chi^2$  analysis<sup>1)</sup>. The results are set out in Table 14.

The fact that the groups were so small might be responsible for the non-significant associations. However, if the hyperlipaemics and "others" are combined into a single group, all its associations will be significant. Hence it seems justified to assume that in the individual subject there exist associations between cholesterol or phospholipids and glyceride-glycerol in the sense that a lower value of cholesterol or phospholipids can be expected to

1) Both series of the observations are put into co-ordinated system and both tables are divided into two equal groups. The resulting 4 groups can be described as  $\begin{matrix} a & b \\ c & d \end{matrix}$  where  $a+b=c+d$  and  $a+d=b+c$  calculated  $\chi^2$

$$\chi^2 = \frac{(ad-bc)^2}{(a+b)(c+d)(a+c)(b+d)}$$

and the coefficient of association ( $\phi$ ) is calculated

$$\phi = \frac{\chi^2}{(a+b+c+d)}$$

## CHAPTER VI

### FREQUENCY ANALYSES OF CHANGES IN SERUM LIPID FACTORS AT THE FAT TOLERANCE TEST

In order to select relationships it might be interesting to analyze, a pilot study was made with respect to the frequencies of changes in the turbidity as well as glyceride-glycerol, cholesterol and phospholipid levels of serum after the fat meal. This frequency study was performed on the  $S_0-S-S$  Series (Table 15) which was divided into three main groups: (A) controls, (B) hypercholesterolemics and hyperlipaemias without and (C) all patients with clinical atherosclerosis. Within each of these groups the frequency of rises after 4 and 7 hours as well as the frequency of decreases from the 4th to the 7th hour were calculated for each lipid factor (Table 15).

Four hours after the fat meal the turbidity and the glyceride-glycerol level of serum had risen in 100 per cent of all groups. The corresponding percentages for cholesterol rises ranged from 61 to 71 per cent. The phospholipid level rose in 100 per cent of Group A 93 per cent of Group B and 91 per cent of Group C. Accordingly it is of interest to investigate the changes in turbidity glyceride-glycerol and phospholipids 4 hours after the fat meal.

While the serum turbidity was higher 7 hours after the fat meal than before it in practically 100 per cent of all three groups, the corresponding proportions for serum glyceride-glycerol increases ranged from 68 to 81 per

TABLE 15. Frequency (in %) of relative changes turbidity glyceride-glycerol cholesterol and phospholipids in serum immediately before and 4 and 7 hours after the fat meal

	$S > S_0$			$S_4 > S_0$			$S > S_7$		
	A	B	C	A	B	C	A	B	C
Turbidity	100	100	100	93	96	100	77	81	89
Glyceride-glycerol	100	100	100	89	79	81	93	71	74
Cholesterol	61	71	69	27	61	53	73	54	44
Phospholipids	100	93	81	93	93	88	89	50	47

$S_0$ ,  $S_4$  and  $S_7$  = Serum immediately before and 4 and 7 hours after the fat meal.

A = Controls = 44.

B = Hypercholesterolemics and hyperlipaemias without clinical atherosclerosis = 24.

C = Normocholesterolemics, hypercholesterolemics and hyperlipaemias with clinical atherosclerosis = 32.

Most workers have reported no or negligible serum cholesterol alterations in response to a fat load (BLOOR, 1910 HILLER *et al* 1924 BLIX 1925 HIRSCH & CARBONARO 1950 POMERANZE BEINFELD & CHESIN 1954 ALBRINK & MAN 1956). The present observation that serum cholesterol alterations after fat loading depend upon whether the individual's fasting cholesterol lies high or low in his own range of serum cholesterol variation implies that one should not expect any simple reproducibility of the serum cholesterol change in response to duplicate fat tolerance tests while the serum cholesterol level of some persons rises that of others falls.

It is known that after a fat meal the serum phospholipid rise (MAN & GILDEA 1932 POMERANZE BEINFELD & CHESIN 1954 HAVEL 1957 *etc*) and this is borne out by the results of the present investigation. In analogy with the case of cholesterol in serum it holds true for the individual subject that the lower the initial serum phospholipid level the higher it rises after a fat meal. The fasting serum with the lower phospholipid level also exhibits the lower glyceride glycerol content. Conversely the lower fasting glyceride-glycerol value showed the smaller increment 4 hours after the fat meal. Supposing that the absorption from the digestive tract is similar at the duplicate fat tolerance tests this would mean that the removal of neutral fats from the blood was more rapid in Group A. According to HAVEL (1957) the phospholipid content rises in the chylomicrons and in the very low

density lipoproteins during the lipaemic phase and in the high density lipoproteins during the postlipaemic phase. The latter rise could conceivably take place more rapidly during a high rate of removal of neutral fats from the blood and this could result in a higher total phospholipid increase 4 hours after a fat meal.

When the individual subjects diet on the two occasions for the duplicate fat tolerance tests was unchanged, changes in the fasting serum levels of glyceride-glycerol cholesterol phospholipids and turbidity of serum usually were in the same direction. With respect to glyceride-glycerol and turbidity this seems to mean that equilibrium obtains between visible fats (chylomicrons and very low density lipoproteins capable of producing turbidity) and invisible fats.

It was true of the whole series that the higher the glyceride-glycerol level of 4-hour serum the greater the chylomicron phase of such serum. Significant associations existed between the turbidity and the glyceride-glycerol content of the chylomicron phase of 4 hour serum as well as between the glyceride glycerol content of the chylomicron phase of 4 hour serum and the turbidity of such serum. But the association did not extend so far as to include the turbidity and the glyceride glycerol level of 4 hour serum. Consequently it may be said that turbidity differences between 4-hour sera in duplicate fat tolerance tests are not a measure of changes in the glyceride glycerol content of such sera.

## CHAPTER VII

### THE FAT TOLERANCE TEST IN VARIOUS GROUPS OF SUBJECTS

#### Serum lipids and turbidity in the control groups

The key role played by the control groups in these analyses justifies some preliminary comments on these groups.

Means and standard deviations for the glyceride-glycerol level and turbidity of serum immediately before as well as 4 and 7 hours after the fat meal in the controls are given in Table 16. The glyceride-glycerol level as well as the turbidity are skewed distributed (cf Fig 6). Conversion of these values into logarithms yields a distribution which is accepted as normal. Anti logarithms for the dispersion (mean  $\pm$  s.e.) are given in the table (i.e. 95 % ranges). The mean glyceride-glycerol

levels and turbidities of male and female controls do not differ significantly. The fact that the glyceride-glycerol values tended to rise before the age of 50 years in the control group as a whole suggests that age might be a factor here. So the glyceride-glycerol was studied in age-matched groups ( $n = 27$ ) of male and female controls but no significant difference emerged.

In fasting serum positive and significant correlations exist between phospholipids and glyceride-glycerol as well as between turbidity and glyceride-glycerol but apparently not between cholesterol and glyceride-glycerol nor between either cholesterol or phospholipids and turbidity.

TABLE 16. *Glyceride-glycerol and turbidity in serum of controls 0, 4 and 7 hours after the fat meal*

		Glyceride-glycerol mMol/l		Turbidity	
		Mean	95% range	Mean	95% range
<i>Men</i>					
	0 hrs				
	4 hrs	1.14	0.88-2.06	0.10	0.03-0.24
	7 hrs	2.01	0.88-4.11	0.23	0.18-1.14
	7 hrs	1.37	0.47-3.33	0.21	0.04-0.80
<i>Women</i>					
	0 hrs				
	4 hrs	1.50	0.48-1.91	0.09	0.02-0.18
	7 hrs	1.87	0.90-2.11	0.42	0.12-1.00
	7 hrs	1.37	0.44-2.40	0.26	0.07-0.69

cent The serum cholesterol level was lower 7 hours after than before the fat meal in approximately 75 per cent of Group A No corresponding changes in serum cholesterol could be demonstrated in Groups B and C (39 and 47%) The serum phospholipids were higher 7 hours after than before the fat meal in from 88 to 98 per cent of Groups A B and C Accordingly it is of interest to study the changes in serum turbidity glyceride-glycerol cholesterol and phospholipids 7 hours after the fat meal

In 77 81 and 89 per cent of Groups A B and C the serum turbidity fell significantly from the 4th to the 7th hour after the fat meal These frequencies were not significantly unequal At 7 hours the serum turbidity was higher than at 4 hours in 3 of the 24 male and 7 of the 20 female controls i.e. in 23 per

cent of Group A Concomitantly 2 men and no woman showed a higher serum glyceride-glycerol level The glyceride-glycerol level fell from the 4th to the 7th hour after a fat meal in 95 per cent of Group A The corresponding proportions of Groups B and C were significantly lower (71 and 74%) From the 4th to the 7th hour after the fat meal a serum cholesterol reduction was noted in 73 per cent of Group A as against 54 per cent of Group B and 44 per cent of Group C This proportion of the controls (Group A) significantly exceeds that of the atherosclerotic subjects (Group C)

The serum phospholipids decreased in 69 per cent of Group A (significant) but showed only random variations in Groups B and C The frequency in Group A was significantly higher than that in Group C

TABLE 17 Distribution of serum glyceride-glycerol before ( $x$ ) and 4 hrs. after the fat meal ( $y$ ) in relation to regression line for the controls  $\bar{y} = 1.56x$ . The observed frequency over regression line compared with the expected frequency (50%)

Group	Number				
	under	over	total	sign. on 5% level	$t^1$
<i>Men</i>					
Hypercholesterolemics without clinical atherosclerosis	4	7	11	no	+0.27
Hypercholesterolemics with clinical atherosclerosis	10	18	28	no	+0.18
Hyperlipaemics without clinical atherosclerosis	8	6	14	no	+0.97
Hyperlipaemics with clinical atherosclerosis	3	3	6	no	+0.89
Ketocholesterolemics normo-glyceridemic atherosclerosis	3	11	14	yes	+0.33
<i>Women</i>					
Hipercholesterolemics without clinical atherosclerosis	13	13	26	no	+0.33
Hypercholesterolemics with clinical atherosclerosis	9	9	18	no	+0.33
Hyperlipaemics without clinical atherosclerosis	6	0	6	yes	+1.49
H. perlipaemics with clinical atherosclerosis	4	4	8	no	+1.49

$t^1$  = correlation between serum glyceride-glycerol before and 4 hours after the fat meal estimated according to  $\sqrt{\frac{r^2}{n}}$

The table reveals that this association fitted the regression equation  $\bar{y} = 1.56x$  in all but two of the groups. When 95 per cent confidence limits are estimated allowance must be made partly for the fact that the variance of  $y$  was not constant and partly for the non normal distribution of the  $y$  values about the line of regression. On

the assumption that the logarithms of all  $y$ 's corresponding to a given  $x$  are normally distributed the estimated limiting functions bounding the area where 95 per cent of the population falls will be (cf Fig 6).

$$\bar{y} = 1.11x$$

$$\bar{y} = 2.56x$$



Serum lipids 4 hours after and immediately before the fat meal

*Glyceride-glycerol* Preliminary observations (ANGERVALL, 1950) suggested that the fat meal caused the serum glyceride-glycerol to rise more the higher the glyceride glycerol level immediately before the fat meal. In an individual subject moreover duplicate fat tolerance tests disclosed an association between the serum glyceride-glycerol levels 4 hours after and immediately before the fat meal (p. 20).

The nature of the association between the serum glyceride glycerol levels immediately before ( $S_0$ ) and 4 hours after ( $S_4$ ) the fat meal — in the controls was studied by evaluating the expression

$$S_4 = \alpha + \beta S_0$$

A linear relationship is assumed between the  $S_4$  and  $S_0$  values. The relationship can be written

$$\bar{y} = \alpha + \beta x$$

where  $\alpha$  and  $\beta$  are constants,  $\bar{y}$  is the conditional mean value of  $S_4$  and  $x$  is the value of  $S_0$ .  $\alpha$  and  $\beta$  are estimated in the usual manner (of SNEDCOR, p. 108). The hypotheses  $\alpha = 0$  and  $\beta = 0$  are tested. If the hypothesis  $\alpha = 0$  is accepted then the ratio of  $y$  and  $x$  is an estimate of the relationship between  $S_4$  and  $S_0$ . If the hypothesis  $\beta = 0$  is rejected by a test at the 5 per cent level the relationship is considered significant. The test is performed as a  $t$

test where  $t = \frac{b}{s_b}$  ( $b$  is an estimate of  $\beta$  and  $s_b$  the standard deviation of  $b$ )

In order to compare two lines e.g. the test of the hypotheses  $\alpha_1 = \alpha_2$  and  $\beta_1 = \beta_2$  the analysis has been performed as an analysis of covariance (as described by SNEDCOR, p. 395). For men the estimate of the coefficient of  $x$  was

$$b_1 = 1.69$$

and for women it was

$$b_2 = 1.40$$

But as  $(b_1 - b_2)$  did not deviate significantly from zero (i.e. there was no statistically significant difference in the  $S_4/S_0$  ratio between men and women) the estimate

$$b = 1.55$$

was found for the entire control group. Because  $b = 1.55 \neq 0$  the association between  $S_4$  and  $S_0$  is significant (on the 5% level).

Considering that the glyceride glycerol values could not be regarded as having a normal distribution the control group was compared with the other groups according to the following principles. The number of observations falling above and below the regression line  $\bar{y} = 1.55x$  were calculated for each group whereupon the observed frequency above the line was compared with the expected frequency of 50 per cent. If no difference significant on the 5 per cent level could be demonstrated the hypothesis was accepted that the group under consideration exhibited the same association between  $S_4$  and  $S_0$  as the control group. The results of this analysis are presented in Table

TABLE 17 *Distribution of serum glyceride-glycerol before (x) and 4 hrs. after the fat meal (y) in relation to regression line for the controls  $y = 1.85x$ . The observed frequency over regression line compared with the expected frequency (50%)*

Group	Number				
	under	over	total	sign. on 5% level	$\chi^2$
<i>Males</i>					
Hypercholesterolaemia without clinical atherosclerosis	4	7	11	no	+0.97
Hypercholesterolaemia with clinical atherosclerosis	10	10	20	no	+0.18
Hypertriglyceridaemia without clinical atherosclerosis	8	6	14	no	+0.97
Hypertriglyceridaemia with clinical atherosclerosis	8	3	11	no	+0.80
Normocholesterolaemia normo-glyceridaemia atherosclerosis	8	11	19	yes	+0.38
<i>Females</i>					
Hypercholesterolaemia without clinical atherosclerosis	13	13	26	no	+0.28
Hypercholesterolaemia with clinical atherosclerosis	9	9	18	no	+0.33
Hypertriglyceridaemia without clinical atherosclerosis	8	0	8	yes	+1.00
Hypertriglyceridaemia with clinical atherosclerosis	4	4	8	no	+1.00

) = correlation between serum glyceride-glycerol before and 4 hours after the fat meal estimated according to  $\sqrt{\frac{x^2}{n}}$

The table reveals that this association fitted the regression equation  $y = 1.85x$  in all but two of the groups. When 95 per cent confidence limits are estimated allowance must be made partly for the fact that the variance of  $y$  was not constant and partly for the non-normal distribution of the  $y$  values about the line of regression. On

the assumption that the logarithms of all  $y$ 's corresponding to a given  $x$  are normally distributed, the estimated limiting functions bounding the area where 95 per cent of the population falls will be (cf. Fig. 6):

$$y = 1.11x$$

$$y = 2.80x$$

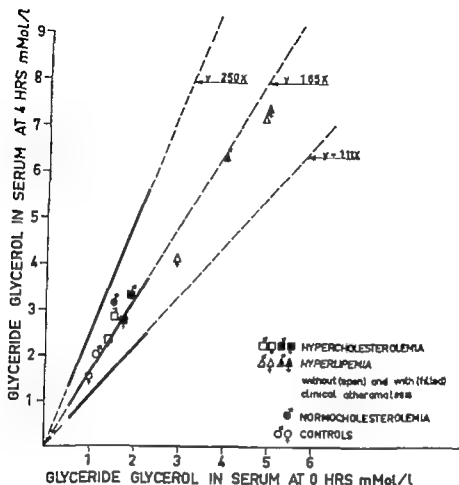


Fig 6 Relation between glyceride-glycerol in serum 4 hours after and before the 1st meal. Heavy lines represent the regression for controls and its 95 per cent confidence limits.

The individual variation is considerable the relative variation about the regression line averages approximately 20 per cent in other words the error of a value  $y_x$  estimated from the line may be up to  $\pm 20$  per cent of  $y_x$ .

With the possible exception of two groups — "male normoglyceridaemic normocholesterolaemics with atherosclerosis" and "female hyperlipaemics without clinical atherosclerosis" — the glyceride glycerol ratio ( $S_4/S_0$ ) did not deviate significantly from that for the controls in any group.

When among all subjects with hyperlipaemia one selects those whose glyceride-glycerol level of fasting serum ( $S_0$ ) exceeds 4 mMol/l one finds that this subgroup has a glyceride glycerol ratio lying significantly below the line  $y = 1.65x$ . This suggests that the relation  $y = 1.65x$  is valid only up to a limiting  $S_0$  value of some 4 mMol/l whereupon the line presumably curves towards the abscissa.

As a supplement to the foregoing analyses, the frequency of extreme values was studied. The results are

TABLE 18. The frequency of extremes of the quotient between glyceride-glycerol in serum 4 hrs. after (y) and before (x) the fat meal

Sex	Group	y/x		
		<1.11)	1.11-2.80	>2.80)
Male	Controls	1	29	2
	Hypercholesterolaemias without clinical atherosclerosis	0	9	2
	Hypercholesterolaemias with clinical atherosclerosis	1	26	1
	Hyperlipaemias without clinical atherosclerosis	1	11	2
	Hyperlipaemias with clinical atherosclerosis	0		1
	Normoglyceridaemia, normocholesterolaemic atherosclerosis	0	10	2
Female	Controls	8	26	0
	H percholesterolaemias without clinical atherosclerosis	1	22	2
	H percholesterolaemias with clinical atherosclerosis	2	13	0
	H perlipaemias without clinical atherosclerosis	0	6	0
	Hyperlipaemias with clinical atherosclerosis	1	6	0

) The range 1.11-2.80 corresponds to the recovered mean  $\pm 2$  of the regression of  $\log y$  upon  $\log x$  because of nonnormal distribution of y about the regression line  $y = 1.63x$

presented in Table 18. The table reveals that 4-hour serum had a glyceride-glycerol level exceeding ...80 times that of fasting serum in 12 of 117 males and in 1 of 93 females. The difference between men and women is significant ( $\chi^2 > 3.84$ ) even after elimination of the group of normoglyceridaemic normocholesterolaemias with atherosclerosis (wherein males only are represented)

Summing up it may be remarked that the glyceride-glycerol level of serum 4 hours after the fat meal is similarly related to that immediately before it in all groups with and without clinical atherosclerosis, perhaps with the exception of normoglyceridaemic normocholesterolaemic men with atherosclerosis, hyperlipaemic women without clinical atherosclerosis, and

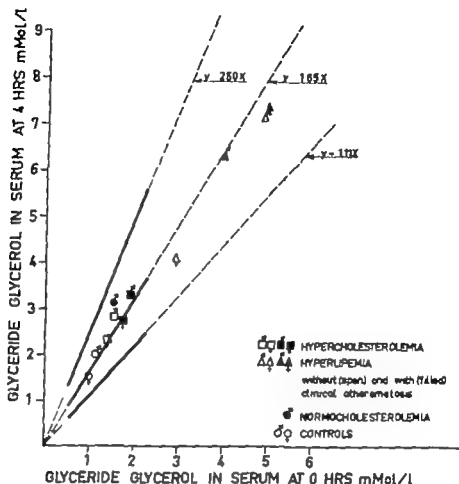


Fig 0 Relation between glyceride-glycerol in serum 4 hours after and before the fat meal. Heavy lines represent the regression for control and its 91 per cent confidence limits.

The individual variation is considerable the relative variation about the regression line averages approximately 20 per cent in other words the error of a value  $y$  estimated from the line may be up to  $\pm 20$  per cent of  $y$ .

With the possible exception of two groups — male normoglycaemic normocholesterolaemics with atherosclerosis and "female hyperlipaemics without clinical atherosclerosis" — the glyceride-glycerol ratio ( $S_4/S_0$ ) did not deviate significantly from that for the controls in any group.

When among all subjects with hyperlipaemia one selects those whose glyceride-glycerol level of fasting serum ( $S_0$ ) exceeds 4 mMol/l one finds that this subgroup has a glyceride-glycerol ratio lying significantly below the line  $y = 1.0x$ . This suggests that the relation  $y = 1.0x$  is valid only up to a limiting  $S_0$  value of some 4 mMol/l whereupon the line presumably curves towards the abscissa.

As a supplement to the foregoing analyses the frequency of extreme values was studied. The results are

TABLE 1 The frequency *1* extreme of the quotient between glyceride-glycerol serum 4 hrs. post (y) and before (x) the fat meal

Sex	Group	y		
		<1.11)	1.11—2.60	>2.60)
Males	Controls	1	23	
	Hypercholesterolaemic without clinical atherosclerosis	0	9	
	Hypercholesterolaemic with clinical atherosclerosis	1	26	1
	H. perlipaemic without clinical atherosclerosis	1	11	2
	H. perlipaemic with clinical atherosclerosis	0		1
Females	Normoglyceridaemic normocholesterolaemic normoarteriosclerotic	0	10	3
	Control	0	24	
	H. percholesterolaemic without clinical atherosclerosis	1	23	2
	Hypercholesterolaemic with clinical atherosclerosis	3	1	0
	Hyperlipaemic without clinical atherosclerosis	0	6	0
	Hyperlipaemic with clinical atherosclerosis	1	4	0

) The range 1.11—2.60 corresponds to the converted mean  $\pm 2$  of the regression of  $\log y$  upon  $\log x$  because of asymmetrical distribution of  $y$  about the regression line  $\hat{y} = 1.63x$

presented in Table 1b. The table reveals that 4-hour serum had a glyceride-glycerol level exceeding ...60 times that of fasting serum in 1 of 117 males and in 1 of 95 females. The difference between men and women is significant ( $\chi^2 > 3.84$ ) even after elimination of the group of normoglyceridaemic normocholesterolaemics with atherosclerosis (wherein males only are represented)

Summing up it may be remarked that the glyceride-glycerol level of serum 4 hours after the fat meal is similarly related to that immediately before it in all groups with and without clinical atherosclerosis perhaps with the exception of normoglyceridaemic normocholesterolaemic men with atherosclerosis hyperlipaemic women without clinical atherosclerosis and

those hyperlipaemics whose fasting serum had a glyceride-glycerol level of at least 4 mMol/l. Moreover in the series as a whole the frequency of extremely high (compared with the fasting value) serum glyceride-glycerol levels 4 hours after the fat meal was higher among men than among women.

**Turbidity** No correlation could be demonstrated in any group between the turbidities of sera immediately before and 4 hours after the fat meal (Comparison between turbidities of different groups see p 53 and Table 23).

**Cholesterol and phospholipids** Table 2 p 17 presents cholesterol levels of fasting serum and cholesterol changes 4 hours after the fat meal. In 9 of 11 groups the cholesterol levels on the latter occasion tended to be higher than on the former. All groups include a majority of subjects with cholesterol rises, the frequency of rises in the entire series being 83 per cent ( $n = 212$ ) which significantly exceeds the 50 per cent expected from random variation. As a rule however the cholesterol changes fell within the range of the standard error of the method and the mean cholesterol change for the controls exhibited no correlation to either age, stature or body surface area.

**Serum phospholipid rises 4 hours after the fat meal** were the rule. The mean rises in the various groups are presented in Table 2 p 17 which shows that the mean phospholipid change was positive and lay between 17 and 36 mg%. The mean phospholipid change was significant for all groups. No difference could be demonstrated between

men and women or between subjects with and without clinical atherosclerosis. No correlation could be detected in any group between the serum phospholipid level immediately before and 4 hours after the fat meal. A positive association was demonstrated between absolute phospholipid rises and relative glyceride-glycerol rises after the fat meal in the control and hypercholesterolaemic groups but not in the groups of hyperlipaemics and normoglycaemic normocholesterolaemics with clinical atherosclerosis. This correlation is significant for both men and women and men plus women among the controls and hypercholesterolaemics.

Serum lipids 7 hours after and immediately before the fat meal

**Glyceride-glycerol** The regression of the serum glyceride-glycerol level 7 hours after the fat meal ( $y$ ) upon that immediately before it ( $x$ ) for male (1) and female (2) controls had the following equations

$$(1) \quad y = 1.12x + 0.03 \quad n = 24 \\ s_y = 0.11 \quad s_{y,x} = 0.400 \quad r = 0.80$$

$$(2) \quad y = 1.5x - 0.04 \quad n = 20 \\ s_y = 0.117 \quad s_{y,x} = 0.225 \quad r = 0.92$$

As no significant difference was present between the regression lines the corresponding regression was analyzed in the control group as a whole the equation being

$$y = 1.1x + 0.01 \quad n = 44 \\ s_y = 0.102 \quad s_{y,x} = 0.310 \quad r = 0.8$$

The observations were normally distributed about the regression line. The constant in the equation is not signi-

significantly different from zero. The coefficient of regression differs significantly from zero but not from unity implying that the association between the serum glyceride-glycerol levels 7 hours after and immediately before the fat meal was statistically significant. Thus generally speaking in the controls the serum glyceride-glycerol level 7 hours after the fat meal was substantially equal to that immediately before it.

Data on the occurrence of positive extremes in the various groups are presented in Table 10 revealing that 7 of 9 groups exhibited significantly higher frequencies of extremes than the controls. The exception were the group of hypercholesterolaemic females without clinical atherosclerosis with no

extremes and the group of normocholesterolaemic normoglyceridaemic atherosclerotics. The hypercholesterolaemic plus hyperlipaemic groups with and without clinical atherosclerosis displayed no significant difference in the frequency of extremes.

When the subjects were classified by serum glyceride-glycerol levels immediately before the fat meal up to and over  $\text{mMol/l}$  and their distribution about the line  $y = 1.17x$  was studied it appeared that whereas 4 observations fell above and 42 below the line in the class up to  $\text{mMol/l}$  21 observations fell above and 9 below the line in the class over  $\text{mMol/l}$ , the inter-class difference being significant.

Analysis of extremes yielded similar results. While 9 of 85 observations up

TABLE 10. Glyceride-glycerol in serum 0 and 7 hours after the fat meal. Comparison between the frequency of extremes

Groups	Without clinical atherosclerosis			With clinical atherosclerosis		
	Mean $\pm 2$	> mean $+2$	$\chi^2$ test	Mean $\pm 2$	> mean $+2$	$\chi^2$ test
Controls, men	23)	1		—	—	—
Controls, women	20	2	—	—	—	—
II percholesterolaemic						
men	4	3	sign.	11	3	sign.
women	11	0		12)	3	sign.
Hyperlipaemic						
men	3	3	sign.	2	2	sign.
women	2	2	sign.	0	4	sign.
Normo(g)lyceridaemic normocholesterolaemic	—	—	—	1	2	—

The dispersion ( ) about the regression line for the whole control group  $y = 1.17x$  = 0.31  $\text{mMol/l}$  extremes = observations more than 2 above this line.

1) Includes values more than 2 below the regression line.



TABLE 20 Regression of serum glyceride-glycerol 7 hours after (y) upon that immediately before (x) the fat meal

Group	Sex		Regression equation	$r_b$	$r$	
Controls	♂+♀	44	$y = 1.17x + 0.01$	0.102	0.210	0.87
Hypercholesterolaemia without clinical atherosclerosis	♂+♀	18	$y = 1.96x - 0.95$	0.508	0.723	0.63
Hypercholesterolaemia with clinical atherosclerosis	♂+♀	26	$y = 0.57x - 0.16$	0.432	1.021	0.56
Hyperlipaemia without clinical atherosclerosis	♂+♀	10	$y = 0.91x + 1.31$	0.111	0.74	0.94
Hyperlipaemia with clinical atherosclerosis	♂+♀	8	$y = 72x - 4.34$	0.233	1.01	0.9
Normocholesterolaemic normo glyceridaemic atherosclerotics	♂+♀	9	$y = 1.28x - 0.10$	0.323	0.484	0.80
Hypercholesterolaemia	♂+♀	44	$y = 1.69x - 0.46$	0.33	1.104	0.61
Hyperlipaemia	♂+♀	18	$y = 1.60x - 0.33$	0.258	1.11	0.82
Total	♂+♀	115	$y = 1.43x + 0.04$	0.071	1.166	0.88

to 2 mMol/l fell outside 2 standard deviations 17 of 30 over 2 mMol/l did so. These proportions are significantly unequal. In other words if the serum glyceride-glycerol level immediately before the fat meal exceeds 2 mMol/l the corresponding value 7 hours after the fat meal will be proportionately higher than if the former is lower. Within these two classes there was no difference between subjects with and without clinical atherosclerosis.

Since the regression lines for male and female controls did not deviate significantly corresponding groups of males and females were combined in the subsequent analysis. The regression

equations for the various groups are presented in Table 20. Statistically the regressions for the hypercholesterolaemic groups with and without clinical atherosclerosis did not deviate so these groups were combined. So were the hyperlipaemic groups because they were deemed too small to justify any assessment of biological differences. As a result the following equations for the regression of the serum glyceride glycerol level 7 hours after the fat meal (y) upon that immediately before it (x) were obtained for the hypercholesterolaemics (1), hyperlipaemics (2) and normocholesterolaemic normoglyceridaemic atherosclerotics (3).

$$(1) \quad \bar{y} = 1.69x - 0.46 \quad n = 44 \\ s = 0.322, s_{\bar{y}} = 1.164 \quad r = 0.61$$

$$(2) \quad \bar{y} = 1.68x - 0.32 \quad n = 18 \\ s = 0.258, s_{\bar{y}} = 1.128 \quad r = 0.82$$

$$(3) \quad \bar{y} = 1.22x - 0.16 \quad n = 9 \\ s = 0.33, s_{\bar{y}} = 0.466 \quad r = 0.89$$

None of these regression lines deviates significantly from that for the controls. For the series as a whole the following equation was obtained

$$\bar{y} = 1.43x + 0.04 \quad n = 115 \\ s = 0.271, s_{\bar{y}} = 1.166 \quad r = 0.86$$

where 0.04 does not differ from zero but 1.43 deviates significantly from unity

Summing up it may be said that the serum glyceride-glycerol level 7 hours after the fat meal is strongly dependent on that immediately before it. For male plus female controls the former can be estimated at 1.17 times the latter. The non-controls exhibited higher frequencies of positive extreme values. With the possible exception of hypercholesterolaemic women no difference was found between subjects with and without clinical atherosclerosis.

**Turbidity** No correlation could be demonstrated between turbidities of sera before and 7 hours after the fat meal. See further p. 53 and Table 23.

#### *(Cholesterol and phospholipids)*

Seven hours after the fat meal the serum cholesterol had fallen  $8.2 \pm 2$  mg% in the control group as a whole ( $n = 44$ )  $12.0 \pm 3$  mg% in the female controls ( $n = 20$ ) and  $5.3 \pm 3$  mg% in

the male controls ( $n = 4$ ). The first two differences but not the last are significant. No corresponding differences were found in the other groups.

No association could be demonstrated between the magnitude of the phospholipid rise 7 hours after the fat meal and the phospholipid level of fasting serum. In the series as a whole the phospholipid rise was weakly correlated to the glyceride-glycerol rise ( $n = 115$   $r = 0.42$ ). The phospholipid rises in groups with glyceride-glycerol rises up to and over 1 mMol/l were  $17.5 \pm 1.7$  and  $48.1 \pm 6.0$  mg% and are significantly unequal. The correlation between the phospholipid and glyceride-glycerol rises was significant in the latter ( $n = 27$   $r = 0.82$ ) but not in the former group ( $n = 84$   $r = 0.19$ ).

Summing up a phospholipid rise in serum generally persisted 7 hours after the fat meal and was positively associated with the corresponding glyceride-glycerol increase especially when the latter exceeded 1 mMol/l.

#### *Serum lipids 7 and 4 hours after the fat meal*

**Glycerid-Glycerol** Preliminary investigations revealed that the turbidity of serum usually fell from the 4th to the 7th hour after the fat meal suggesting that the removal of lipids from the blood stream exceeded the absorption of lipids from the gastrointestinal tract. In the light of the close association demonstrated between the serum glyceride-glycerol levels 4 hours after and immediately before the fat meal as well as between those

7 hours after and immediately before the fat meal one would expect to find a similar association between the serum glyceride-glycerol levels 7 and 4 hours after the fat meal. One would expect the glyceride-glycerol difference between the 4th and 7th hour to be dependent on the value immediately before the fat meal because  $S_4 = 1.04 \pm S_0$  and  $S_7 = 1.1 \pm S_0$  for the controls so  $S_4 - S_7 = S_0(1.04 - 1.17) = 0.48 S_0$ . No such equality could be demonstrated for the controls ( $\chi^2 = 0$   $n = 44$ ) however and therefore it seemed interesting to analyze the association between the serum glyceride-glycerol levels 7 and 4 hours after the fat meal.

As when the association between the 4-hour and 0 hour values was determined, the regression of the 7 hour glyceride-glycerol level ( $y$ ) upon that 4 hours ( $x$ ) after the fat meal was found for male (1) and female (2) controls

$$\begin{aligned} (1) \quad & \bar{y} = 0.48x + 0.27 \quad n = 24 \\ & s_y = 0.110 \quad s_{y,x} = 0.480 \quad r = 0.67 \\ (2) \quad & \bar{y} = 0.61x - 0.14 \quad n = 20 \\ & s_y = 0.081 \quad s_{y,x} = 0.338 \quad r = 0.91 \end{aligned}$$

The regression coefficient for female controls significantly exceeds that for male (of Fig 7) and the constants differ from zero. The observations are normally distributed about the regression lines.

For the purposes of the subsequent analysis male and female groups and also groups with and without clinical atherosclerosis were kept separate. Since the resulting groups of hyperlipaemias were small they were combined with the corresponding hypercholesterolaemia groups. The regression equations for the various groups thus formed will be found in Table 21 and the regression lines are presented in Fig 7. There was no difference between the male and female groups of hyperlipae-

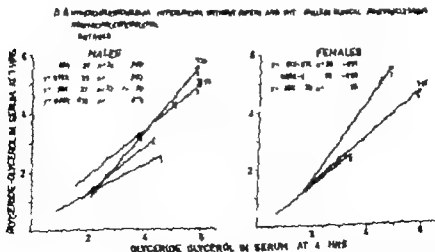


Fig 7 Relation between glyceride-glycerol in serum 4 and 7 hours after the fat meal

TABLE 1 Regression of serum glyceride-glycerol 7 hours after (y) post fat meal 4 hours after (x) the fat meal

Group	Sex	Regressions equation	r
Control	♂	1 $y = 0.45x + 0.27$ $n = 116$ $0.440$ $0.67$	
		20 $y = 0.81x - 0.14$ $n = 101$ $0.234$ $0.91$	
Hypercholesterolaemic + Hypertriglyceridaemic without clinical atherosclerosis	♂	18 $y = 0.78x + 0.20$ $n = 97$ $0.448$ $0.93$	
		13 $y = 0.87x - 0.12$ $n = 107$ $0.857$ $0.84$	
	♂ +	24 $y = 0.82x + 0.01$ $n = 102$ $0.740$ $0.93$	
Hypercholesterolaemic - Hypertriglyceridaemic with clinical atherosclerosis	♂	22 $y = 1.20x - 1.57$ $n = 129$ $0.993$ $0.40$	
		13 $y = 1.67x - 1.23$ $n = 124$ $1.25$ $0.9$	
	♂ +	34 $y = 1.42x - 1.78$ $n = 173$ $0.736$ $0.93$	
Normocholesterolaemic normo- glyceridaemic without clinical atherosclerosis	♂	9 $y = 0.61x + 0.33$ $n = 91$ $0.301$ $0.7$	

mies plus hypercholesterolaemias with out clinical atherosclerosis. But the regression line for the corresponding females with clinical atherosclerosis lay on a significantly higher level than that for the males. Moreover both the latter groups had significantly higher coefficients of regression than the groups with out clinical atherosclerosis. The group of normocholesterolaemic, normoglyceridaemic atherosclerotics had a regression line with higher slope than the male controls but lower slope than other males with clinical atherosclerosis and it was on a lower level than that for hypercholesterolaemias plus hyperlipaemias without clinical atherosclerosis.

This means that in two of three comparisons between corresponding male and female groups the latter exhibited relatively slower fat removal from the blood stream and/or delayed absorption between the 4th and 7th hour after the fat meal. This fat removal also seems slower in groups with than in groups without clinical atherosclerosis.

So far it has been assumed that the regression of the 7 hour glyceride-glycerol values upon the 4 hour values is linear. When the glyceride-glycerol values 4 hours after the fat meal for all the subject ( $n = 116$ ) were classified (see Table 2) it appeared that the regression could be considered linear up to a serum glyceride-glycerol level of approximately 4 mmol/l (a) while the spread was comparatively great for glyceride-glycerol levels exceeding 4 mmol/l (b). The following regression equations were calculated:

- (a)  $y = 0.82x + 0.20$   $n = 73$   
 $s = 0.078$   $s = 0.837$   $r = 0.83$
- (b)  $y = 1.13x - 0.13$   $n = 22$ ,  
 $s = 0.182$   $s = 2.986$   $r = 0.80$

where y and x are the serum glyceride-glycerol level 7 and 4 hours, respectively after the fat meal. The coefficients of regression differ significantly.

Accordingly in the present subjects the association between the serum glyceride-glycerol level 4 and 7 hours

TABLE 22 Relation between serum glyceride-glycerol 7 (8) and 4 (9) hours after the fat meal

	9 classes in mMol/l				
	0-2	2-4	4-6	6-8	8
$\bar{S}$ mean	1.5	2.39	3.43	4.85	8.69
$S_7$ mean	1.08	1.78	2.33	4.68	9.1
$S - S_7$ mean	+0.44	+0.61	+1.10	+0.14	-0.32
$\frac{S - S_7}{S} \times 100$	+29	+26	+32	+3	-6
$n$	32	41	20	13	9
$S > S_7$ $n$		5	4	4	4
$\frac{n}{N} \times 100$	0	12	20	24	44

after the fat meal depended upon whether the 4 hour serum glyceride glycerol level was high or low the boundary being approximately 4 mMol/l. Below this limit the serum glyceride glycerol level fell about 30 per cent from the 4th to the 7th hour after the fat meal and above this limit it fell negligibly or even rose. Thus the 7 hour value exceeded the 4 hour value (i.e. lies above the 45 line in Fig 8) for 11 of 93 subjects in group (a) versus 8 of 22 in group (b) (significant difference). In this respect no difference could be demonstrated between subjects without ( $n = 28$  not including controls) and with ( $n = 43$ ) clinical atherosclerosis either above or below a 4 hour serum glyceride glycerol level of 4 mMol/l. As appears from Table 22 and Fig 8 the frequency of cases with high 7 hour levels in per cent of the class frequency rose from 0 per cent in the class 0-2 mMol/l to some 44 per cent at levels exceeding 6 mMol/l.

On the other hand when the distribution about the regression line for

the controls ( $\bar{y} = 0.68x + 0$  or  $n = 44$   $s_y = 0.076$   $s_{y,x} = 0.146$   $r = 0.74$ ) was studied it appeared that all hyperlipaemics (18 of 18) and 34 of 44 hypercholesterolaemics (14 of 18 with out and 20 of 26 with clinical atherosclerosis) fell above it (significant) versus only 4 of 9 normocholesterol aemic normoglycidaemic atherosclerotics. The results were similar when this analysis was limited to the range of variation exhibited by the controls. Consequently compared with the controls hypercholesterolaemics and hyperlipaemics regardless of whether or not they had clinical atherosclerosis exhibited a higher serum glyceride glycerol level 7 hours after the fat meal in proportion to the 4-hour value.

In sum then the serum glyceride glycerol levels 7 and 4 hours after the fat meal were significantly associated. This association was not the same in these subjects at low 4 hour values (up to 4 mMol/l) and at high 4 hour values (over 4 mMol/l). The demonstrated difference between subjects

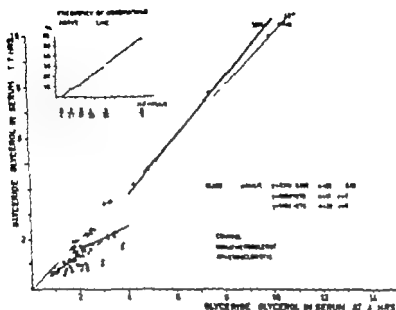


Fig. 4. Relation between glyceride-glycerol in serum 4 and 7 hours after the fat meal

with and without clinical atherosclerosis can to a large extent be accounted for by these differing associations. Regardless of the presence of clinical atherosclerosis hypercholesterolaemia and hyperlipaemia exhibited a higher 7 hour value than the controls in proportion to the 4 hour values.

(Cholesterol and phospholipids) From the 4th to the 7th hour after the fat meal the serum cholesterol level decreased  $8.4 \pm 3.1$  mg% in the male controls and  $13.8 \pm 4.8$  mg% in the female controls, both these differences being significant. The corresponding decreases in serum phospholipids were  $7.3 \pm 3.2$  mg% in male controls (significant),  $6.7 \pm 3.8$  mg% in female controls and  $7.0 \pm 2.3$  mg% in the control group as a whole (signi-

ficant). No similar changes were demonstrated in the other groups for either cholesterol or phospholipids.

#### Comment

Many authors have expressed the view that in fat tolerance tests there is a relationship between atherosclerosis and a prolonged and elevated chylomicron curve (Moreton 1947 1948 1950; Becker Meyer & Necheles 1950) or serum turbidity curve (Schwartz Woldow & Duxworth, 1952; Woldow Chaffin & Evans 1954; Harnitt 1956; Broxte Stewart & Blackburn 1958; Mitchell & Broxte Stewart 1959; Bouchier & Broxte Stewart 1961; Horlick (1956 1957) on the other hand felt that a high fat

TABLE 22. Relation between serum glyceride-glycerol 7 ( $S_7$ ) and 4 ( $S_4$ ) hours after the fat meal

	$S_4$ -classes in mMol/l				
	0—	2—3	3—4	4—5	5
$S_7$ mean	1.52	1.39	2.42	4.82	8.69
$S_7$ mean	1.08	1.78	3.3	4.68	9.1
$S_7 - S_4$ mean	+ 0.44	+ 0.61	+ 1.10	+ 0.14	- 0.41
$\frac{S_7 - S_4}{S_4} \times 100$	+29	+36	+32	+3	-6
$n$	11	41	90	13	0
$S_7 > S_4$	3	5	4	4	4
$\frac{n}{N} \times 100$	0	11	20	34	44

after the fat meal depended upon whether the 4 hour serum glyceride glycerol level was high or low the boundary being approximately 4 mMol/l. Below this limit the serum glyceride glycerol level fell about 30 per cent from the 4th to the 7th hour after the fat meal and above this limit it fell negligibly or even rose. Thus the 7 hour value exceeded the 4 hour value (it lies above the 45 line in Fig. 8) for 11 of 93 subjects in group (a) versus 8 of 22 in group (b) (significant difference). In this respect no difference could be demonstrated between subjects without ( $n = 28$  not including controls) and with ( $n = 43$ ) clinical atherosclerosis either above or below a 4 hour serum glyceride-glycerol level of 4 mMol/l. As appears from Table 22 and Fig. 8 the frequency of cases with high 7 hour levels in per cent of the class frequency rose from 6 per cent in the class 0—2 mMol/l to some 44 per cent at levels exceeding 5 mMol/l.

On the other hand when the distribution about the regression line for

the controls ( $\bar{y} = 0.86x + 0$  or  $N = 44$ ,  $s_y = 0.06$ ,  $s_x = 0.146$ ,  $r = 0.4$ ) was studied it appeared that all hyperlipaemic (18 of 18) and 34 of 44 hypercholesterolaemics (14 of 18 with out and 20 of 26 with clinical atherosclerosis) fell above it (significant) versus only 4 of 9 normocholesterol aemic normoglyceridaemic atherosclerotics. The results were similar when this analysis was limited to the range of variation exhibited by the controls. Consequently compared with the controls hypercholesterolaemics and hyperlipaemics regardless of whether or not they had clinical atherosclerosis, exhibited a higher serum glyceride-glycerol level 7 hours after the fat meal in proportion to the 4 hour value.

In sum then the serum glyceride glycerol levels 7 and 4 hours after the fat meal were significantly associated. This association was not the same in these subjects at low 4 hour values (up to 4 mMol/l) and at high 4 hour values (over 4 mMol/l). The demonstrated difference between subjects

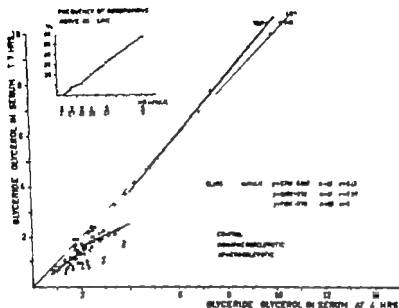


Fig. 8. Relation between glyceride glycerol in serum 4 and 12 hours after the fat meal

with and without clinical atherosclerosis can to a large extent be accounted for by these deviating associations. Regardless of the presence of clinical atherosclerosis, hypercholesterolaemias and hyperlipaemias exhibited a higher hour value than the controls in proportion to the 4 hour values.

**Cholesterol and phospholipids.** From the 4th to the 7th hour after the fat meal the serum cholesterol level decreased  $8.4 \pm 3.1$  mg% in the male controls and  $12.8 \pm 4.8$  mg% in the female controls both these differences being significant. The corresponding decreases in serum phospholipids were  $7.3 \pm 3.8$  mg% in male controls (significant)  $8.7 \pm 3.8$  mg% in female controls and  $7.6 \pm 2.3$  mg% in the control group as a whole (signi-

ficant). No similar changes were demonstrated in the other groups for either cholesterol or phospholipid.

### Comment

Many authors have expressed the view that in fat tolerance tests there is a relationship between atherosclerosis and a prolonged and elevated chylomicron curve (MORETOS 194 1948 1950 BECKER, MEYER & NECKEL 1950) or serum turbidity curve (SCHWARTZ, WOLDOW & DUXSHORE, 1953 WOLDOW CHAPMAN & EVANS 1954 BARRITT 1956 BROXTON-STEWART & BLACKBURN 1958 MITCHELL & BROXTON-STEWART 1950 BOUCHIER & BROXTON-STEWART 1961) HOMLICK (1956 1957) on the other hand felt that a high-fat



test meal failed to uncover lipid metabolic individuals with atherosclerosis (total fatty acids determination) POMERANZE BEINFELD & CHESIN (1954) and KUO JOYNER & REINHOLD (1956) maintained that hypercholesterolaemia and hyperlipaemia as such gave rise to prolonged lipaemia (total fatty acids) The present investigation bears out the latter workers Moreover DEBOROUGH (1963) found that the turbidity as well as the triglyceride level of serum after a fat meal were higher in patients with ischaemic heart disease than in controls and this difference was correlated to differences in the triglyceride level of fasting serum The results of the present investigation are in agreement with this finding

The behaviour of the serum cholesterol after a fat meal agrees well with the findings of HIRSCH & CARBOKARY (1950) POMERANZE BEINFELD & CHESIN (1954) and ALBRINK & MAN (1956) all of whom reported no or negligible cholesterol changes after a fat meal Others have reported increases of the order of 5 per cent (HILLER *et al* 1934 BLOTNER 1935 BRUN 1940 HAVEL 1957 KINGSBURY MORGAN & SHERVINGTON 1960) No corollary to the association between body weight and the cholesterol changes induced in serum by a fat meal in BLOTNER'S (1935) investigation was found in the present study No significant serum cholesterol increase was demonstrated in any group 4 hours after the fat meal Both control groups showed a small but significant cholesterol fall between the 4th and 7th

hour usually to a level below the cholesterol concentration in fasting serum In this respect there was significant difference between the controls and the subjects with clinical atherosclerosis This bears out NIKKILÄ & KONTTINEN (1962) who observed a serum cholesterol decrease 8 hours after a meal of 55 g butter fat

That the serum phospholipids may rise after a fat meal has been reported previously (MAN & GILDEA 1932 POMERANZE BEINFELD & CHESIN 1954 HAVEL 1957 KINGSBURY MORGAN & SHERVINGTON 1960) ALBRINK & MAN (1956) on the other hand found no such rise after a fairly small test meal (60 g fat) The amount of fat ingested seems a significant factor This is illustrated by the small phospholipid increase recorded 3 hours after a meal of 60 g of fat as contrasted with the 18 per cent rise obtained after 3.5 g to 4 g of fat per kg body weight (MAN & GILDEA 1932) The results reported by POMERANZE BEINFELD & CHESIN (1954) TALBOTT & KEATING (1962) and SKINNER & HAUDE (1963) indicate that the greatest alimentary lipaemia is attended by the highest serum phospholipid increase The latter finding is borne out by the present investigation But like serum cholesterol the phospholipids decreased significantly between the 4th and 7th hours after the fat meal in the controls but not in the other group

As appears from Table 6 page the male controls had significantly

higher turbidity of fasting serum than the female and the other groups exhibited similar tendencies. The corresponding serum glyceride-glycerol levels for males also tended to be higher than for females in all groups. There was a significant difference between the male and female controls in the serum glyceride-glycerol level 4 hours after the fat meal and also between male and female hypercholesterolaemic with clinical atherosclerosis in serum turbidity at 4 hours. Generally speaking therefore in the present series 1 subjects the serum turbidity and glyceride-glycerol variations in response to the fat meal displayed few significant differences between men and women.

Table 23 presents some direct comparisons between the serum turbidity and glyceride-glycerol values observed within groups immediately before 4 hours after and 7 hours after the fat meal. The table reveals that the group of normocholesterolaemic normoglyceridaemic atherosclerotics (males only) in no respect differed significantly from the male controls (but see also p 42) (conversely all groups of hypercholesterolaemics (males and females with and without clinical atherosclerosis) exhibited significant differences from the corresponding controls in regard to the serum glyceride-glycerol level immediately before the fat meal but not to all the 4 hour and 7 hour values. Furthermore Student's *t* was consistently higher for the fasting differences than for the 4-hour and 7 hour differences something characterizing practically all comparisons of

this type made. The corresponding analysis of the turbidity variations in response to the fat meal disclosed a significant difference in the turbidity of fasting serum between female hypercholesterolaemics and control but the differences were on the whole definitely smaller and usually not significant and Student's *t* was usually consistently smaller than the corresponding values for glyceride-glycerol differences and often not significant when the latter were significant.

Neither the glyceride-glycerol level nor the turbidity of serum observed at fat tolerance tests showed any significant differences between the combined groups with and the combined groups without clinical atherosclerosis.

The group presented in the table comprising 10 men with established myocardial infarction all had shown a clinical course and ECG changes typical of this affection. The group included 6 subjects classified as hypercholesterolaemics, 1 hyperlipaemic (with a fasting serum glyceride-glycerol level of 4.66 mllol/l) and 3 normoglyceridaemic normocholesterolaemics. The group of myocardial infarctions differed significantly from the male controls only in the serum glyceride-glycerol level immediately before the fat meal.

The group of subjects with stenosis of the renal artery comprised 5 hypercholesterolaemics (6 males and 3 females) and 3 normo-glyceridaemic normocholesterolaemics. This group differed from the combined control groups with respect to the glyceride-glycerol levels (with the highest values of Stu

TABLE 23 Differences between groups in serum glyceride-glycerol and turbidity before and after the fat meal

Sex	Group	n	Glyceride-glycerol mMol/l			Turbidity		
			S <sub>4</sub>	S	S <sub>4</sub>	S	S	S <sub>4</sub>
Male	Controls	24	1.18	3.34	1.37	0.08	0.57	0.51
	compared with							
	Normocholesterolaemic normo- glyceridaemic atherosclerotics	9	1.50	3.06	1.81	0.08	0.51	0.54
	t		1.33	1.27	1.40	0.21	0.71	0.53
	Hypercholesterolaemias without clinical atherosclerosis	7	1.61	3.08	2.59	0.13	1.05	0.45
	t		2	1.94	2.30	1.95	1.88	2.20
	Hypercholesterolaemias with clinical atherosclerosis	18	1.82	3.38	2.58	0.09	0.82	0.48
	t		3.74	3.36	2.94	1.11	1.95	1.63
	Myocardial infarction	10	1.93	3.52	2.76	0.10	0.72	0.55
	t		5.12	1.64	2.04	0.89	0.05	1.39
Female	Controls	20	1.05	1.75	1.47	0.06	0.44	0.48
	compared with							
	Hypocholesterolaemias without clinical atherosclerosis	11	1.47	2.38	1.67	0.09	0.67	0.34
	t		2.40	2.44	1.98	3.18	1.37	1.41
	Hypercholesterolaemias with clinical atherosclerosis	8	1.4	2.66	2.7	0.08	0.42	0.1
	t		2.01	2.66	2.74	2.30	0.25	1.94
Male + female	Control	44	1.12	2.01	1.33	0.07	0.51	0.23
	compared with							
	Subject with renal artery stenosis	12	1.7	3.04	2.07	0.09	0.53	0.43
	t		3.92	2.76	2.10	1.15	0.18	1.62
	Hypercholesterolaemias without clinical atherosclerosis	18	1.5	2.65	1.98	0.10	0.8	0.40
	compared with							
	Hypercholesterolaemias with clinical atherosclerosis	22	1.9	3.16	2.64	0.09	0.0	0.48
	t		1.91	1.63	1.61	-0.74	-0.78	0.6
	Hyperlipaemias without clinical atherosclerosis	9	4.17	5.2	5.26	0.21	1.25	0.91
	compared with							
	Hyperlipaemias with clinical atherosclerosis	8	4.06	7.02	8.31	0.50	1.33	1.54
	t		0.50	0.82	1.63	-0.32	0.13	0.97

dent  $s/t$  for the differences in fasting serum) but not to turbidity.

Summarizing it may be inferred from the foregoing that (a) the serum glyceride-glycerol level is better than its turbidity as a criterion for distinguishing between clinical groups; (b) the  $t$  values for the fasting-serum glyceride-glycerol differences are generally higher than those for the 4-hour and 7 hour differences implying that the

degree of statistical significance of the difference between clinical groups produced by fat tolerance tests usually is no higher than that observed between fasting values and (c) hypercholesterolaemics or hyperlipaemics with and without clinical atherosclerosis do not differ significantly in the serum glyceride-glycerol levels and turbidities either before or 4 and 7 hours after the fat meal.

TABLE 23 *Differences between groups in serum glyceride-glycerol and turbidity before and 7 hours after the fat meal*

Sex	Group	n	Glyceride-glycerol mmol/l			Turbidity	
			$\bar{x}$	S	$S_x$	S	$S_x$
Male	Controls	24	1.18	2.24	1.37	0.08	0.37
	compared with						
	Normocholesterolaemic, normo- glyceridaemic atherosclerotics	9	1.50	2.66	1.81	0.08	0.51
	t		1.33	1.2	1.40	0.1	0.71
	Hypercholesterolaemias without clinical atherosclerosis		1.61	3.06	2.59	0.13	1.03
	t		2.7	1.94	2.30	1.95	1.88
	Hypercholesterolaemias with clinical atherosclerosis	19	1.82	3.35	2.68	0.09	0.82
Female	Myocardial infarction	10	1.93	3.22	2.76	0.10	0.92
	t		5.12	1.84	2.04	0.89	0.95
	Control	20	1.03	1.75	1.27	0.06	0.44
	compared with						
	Hypercholesterolaemias without clinical atherosclerosis	11	1.4	2.38	1.67	0.08	0.67
	t		2.80	2.44	1.98	2.19	1.37
	Hypercholesterolaemias with clinical atherosclerosis	8	1.4	2.66	1.7	0.08	0.43
Male + female	t		3.01	3.56	2.74	2.30	0.25
	Controls	44	1.12	0.1	1.32	0.07	0.51
	compared with						
	Subjects with renal artery stenosis	1	1.72	2.94	2.07	0.09	0.53
	t		2.0*	2.76	2.10	1.15	0.18
	Hypercholesterolaemias without clinical atherosclerosis	18	1.32	1.63	1.88	0.10	0.82
	compared with						
	Hypercholesterolaemias with clinical atherosclerosis	36	1.9	2.16	2.64	0.09	0.0
Hyperlipaemia without clinical atherosclerosis	t		1.93	1.63	1.61	-1.74	-0.78
	Hyperlipaemia without clinical atherosclerosis	9	4.1	5.82	5.6	0.21	1.13
	compared with						
	Hyperlipaemia with clinical atherosclerosis	8	4.68	0*	8.31	0*0	1.25
	t		0.50	0.83	1.63	-0.32	0.13
							0.9

dent  $s$  ( for the differences in fasting serum) but not to turbidity.

Summarizing it may be inferred from the foregoing that (a) the serum glyceride-glycerol level is better than its turbidity as a criterion for distinguishing between clinical groups; (b) the  $t$  values for the fasting-serum glyceride-glycerol differences are generally higher than those for the 4 hour and hour differences, implying that the

degree of statistical significance of the difference between clinical groups produced by fat tolerance tests usually is no higher than that observed between fasting values; and (c) hypercholesterolaemics or hyperlipaemics with and without clinical atherosclerosis do not differ significantly in the serum glyceride-glycerol levels and turbidities either before or 4 and 7 hours after the fat meal.

TABLE 23 Differences between groups in serum glycerol-glycerol and turbidity before and after the fat meal

Sex	Group	n	Glyceride-glycerol mMol/l			Turbidity		
			S	S	S	S	S	S <sub>T</sub>
Male	Controls	4	1.18	2.24	1.37	0.04	0.5	0.1
	compared with							
	normocholesterolaemic normo- glyceridaemic atherosclerotics	9	1.50 1.33	2.66 1.27	1.81 1.40	0.08 0.31	0.51 0.71	0.24 0.53
	Hypercholesterolaemics without clinical atherosclerosis	7	1.61 —	3.08 1.94	.59 2.30	0.13 1.93	1.05 1.88	0.48 2.20
	Hypercholesterolaemics with clinical atherosclerosis	18	1.62 3.74	3.38 2.36	2.66 2.94	0.08 1.11	0.82 1.93	0.46 1.63
	Myocardial infarction	10	1.93 5.1	3.22 1.84	2.76 2.04	0.10 0.89	0.7 0.95	0.53 1.30
Female	Controls	20	1.03	1.75	1.17	0.06	0.44	0.26
	compared with							
	Hypercholesterolaemics without clinical atherosclerosis	11	1.4 2.80	2.38 2.44	1.67 1.98	0.08 2.19	0.67 1.37	0.34 1.41
	Hypercholesterolaemics with clinical atherosclerosis	8	1.4 3.01	2.66 2.66	.77 .74	0.08 1.30	0.42 0.23	0.51 1.90
Males + female	Control	44	1.12	.01	1.32	0.07	0.51	0.23
	compared with							
	Subject with renal artery stenosis	1	1.7 2.02	2.04 2.76	1.07 1.0	0.09 1.15	0.63 0.18	0.43 1.6
	Hypercholesterolaemics without clinical atherosclerosis	18	1.5	2.63	1.98	0.10	0.82	0.40
	compared with							
	Hypercholesterolaemics with clinical atherosclerosis	28	1.9 1.93	3.10 1.63	2.64 1.61	0.09 -1.74	0.0 -0.78	0.48 0.67
	Hyperlipaemics without clinical atherosclerosis	9	4.1	5.8	1.20	0.31	1.23	0.91
	compared with							
	Hyperlipaemics with clinical atherosclerosis	8	4.66 (0.59)	7.02 0.62	1.31 1.63	0.20 -0.32	1.32 0.13	1.34 0.67

dent  $s/t$  for the differences in fasting serum) but not to turbidity

Summarizing it may be inferred from the foregoing that (a) the serum glyceride-glycerol level is better than its turbidity as a criterion for distinguishing between clinical groups; (b) the  $t$  values for the fasting-serum glyceride-glycerol differences are generally higher than those for the 4 hour and 7-hour differences implying that the

degree of statistical significance of the difference between clinical groups produced by fat tolerance tests usually is no higher than that observed between fasting values and (c) hypercholesterolaemics or hyperlipaemics with and without clinical atherosclerosis do not differ significantly in the serum glyceride-glycerol levels and turbidities either before or 4 and 7 hours after the fat meal.



# GLYCERIDE GLYCEROL LEVEL AND TURBIDITY OF SERUM OF ITS SUBNATANT AND ITS CHYLOMICRON PHASE

The glyceride-glycerol and turbidity of serum its subnatant and its chylomicron phase (after centrifugation at 25000 g for 1 hour) immediately before and 4 hours after the fat meal were determined in 106 subjects and in 52 subjects also 7 hours after the fat meal all subjects being on ordinary diet. The results are set out in Table 7 p. 23

## Glyceride glycerol of whole fasting serum and its subnatant

*Controls* The glyceride-glycerol content of fasting serum differed significantly from that of its subnatant in both male and female controls the respective mean differences being  $0.08 \pm 0.03$  and  $0.03 \pm 0.01$  mMol/l. These differences which do not deviate significantly represent the glyceride-glycerol of the chylomicron phase of fasting serum.

The regressions of the glyceride-glycerol content of the subnatant ( $y$ ) upon that of whole serum ( $x$ ) in male (1) and female (2) controls were as follows

- (1)  $y = 0.81x + 0.15 \quad n = 38$   
 $s_y = 0.062 \quad s_{y,x} = 0.008 \quad r = 0.01$
- (2)  $y = 1.01x - 0.04 \quad n = 34$   
 $s_y = 0.030 \quad s_{y,x} = 0.063 \quad r = 0.99$

The constants  $+0.15$  and  $-0.04$  both deviate from zero and the regression

coefficient  $0.91$  is less than unity. The lines representing these two equations have different slopes. This suggests that the relationship between the glyceride-glycerol level of fasting serum and that of its subnatant was different in male and female controls.

*Other groups* No significant difference between the glyceride-glycerol of fasting serum and that of its subnatant could be demonstrated in any of the other groups nor in either the combined groups with or the combined groups without clinical atherosclerosis (of Table 7). The table reveals that those groups whose fasting serum had a high glyceride-glycerol level had a larger chylomicron phase than those groups whose corresponding glyceride-glycerol level was low. The controls and the other groups were therefore divided into classes with fasting serum glyceride-glycerol below 1 and between 1 and 2 mMol/l (of Table 24). It appeared that unlike the other groups, the controls showed a significant difference between the glyceride-glycerol levels of whole fasting serum and of its subnatant — the chylomicron phase — in both these classes ( $0.029 \pm 0.004$  and  $0.043 \pm 0.016$  mMol/l respectively). Each of these chylomicron phases also significantly exceeded that in the corresponding class of the other groups.

TABLE 24 *Glyceride-glycerol of fasting serum and its substrate and their difference (chylomicron phase)*

		Serum glyceride-glycerol in mVal/l				
		0-1	1-	2-3	3-4	4
Controls	-	32	26		-	-
Bergen		0.74	1.23	1.29		
Substrate		0.71	1.26	1.73		
Chylomicron phase		0.03	0.04	0.36		
Standard error of mean		0.004	0.010	0.470		
Chylomicron phase as per cent of serum		5	3	21		
Others	-	7	10	23	6	10
Bergen		0.84	1.49	1.44	2.40	0.32
Substrate		0.86	1.48	2.36	2.69	4.41
Chylomicron phase		0.019	0.0029	0.093	0.079	1.092
Standard error of mean		0.012	0.016	0.043	0.162	0.610
Chylomicron phase as per cent of serum		2	0	4	1	30
Total series	-	29	36	23	6	1
Bergen		0.78	1.41	1.43	2.40	0.32
Substrate		0.74	1.39	2.31	2.69	4.41
Chylomicron phase		0.023	0.017	0.123	0.279	1.84
Standard error of mean		0.005	0.011	0.035	0.162	0.610
Chylomicron phase as per cent of serum		3	2	5	1	30

# Turbidity and glyceride-glycerol of whole fasting serum and its substrate

It appeared that the turbidity and glyceride-glycerol of fasting serum exhibited a positive and significant correlation in the entire series ( $n = 166$ ,  $r = 0.79$ ) as well as in three of its eleven subgroups (Table 26)

The regressions of the turbidity ( $y$ ) upon the glyceride-glycerol level ( $x$ ) of fasting serum in the combined groups of hypercholesterolaemias and hyperlipaemias with (1) and without (2) clinical atherosclerosis were (Fig 5)

$$(1) \quad y = 0.052x + 0.029 \quad n = 45 \\ s = 0.0089 \quad s_y = 0.0084 \quad r = 0.90$$

$$(2) \quad y = 0.042x + 0.017 \quad n = 43 \\ s = 0.040 \quad s_y = 0.0463 \quad r = 0.95$$

While the regression coefficients differ significantly the constants do not deviate from zero. This analysis shows that the group without clinical atherosclerosis had relatively higher

) Because of the skew distribution of turbidity ( $y$ ) about the mean, the regression lines of  $\log y$  upon  $(\log \text{glyceride-glycerol})$  were calculated and even then the regressions have differed significantly

TABLE 23. Regression of turbidity ( $y$ ) upon glyceride-glycerol ( $x$ ) before ( $S_0$ ) and 4 hours after ( $S_1$ ) the fat meal

Group	Sex	n	Regression equation $\begin{cases} S \\ S_1 \end{cases}$	$s_b$	$s_{b \cdot x}$	r
Control	♂	26	$\begin{cases} y = 0.012x + 0.072 \\ y_x = 0.002x + 0.063 \end{cases}$	0.00546 0.0231	0.0468 0.164	0.18 0.4
	♀	34	$\begin{cases} y = 0.009x + 0.084 \\ y = 0.31x + 0.081 \end{cases}$	0.00330 0.0745	0.0358 0.254	0.09 0.43
Hypercholesterolaemia without clinical atherosclerosis	♂	8	$\begin{cases} y = 0.003x + 0.133 \\ y = 0.40x - 0.13 \end{cases}$	0.03495 0.1166	0.0598 0.260	-0.03 0.00
	♀	22	$\begin{cases} y = 0.0004x + 0.089 \\ y = 0.58x - 0.69 \end{cases}$	0.01263 0.0634	0.0747 0.25	0.01 0.81
Hypercholesterolaemia with clinical atherosclerosis	♂	18	$\begin{cases} y = 0.015x + 0.062 \\ y = 0.19x + 0.13 \end{cases}$	0.00301 0.0444	0.0527 0.197	0.46 0.44
	♀	10	$\begin{cases} y = 0.021x + 0.040 \\ y = 0.12x + 0.18 \end{cases}$	0.00520 0.0620	0.0108 0.187	0.78 0.51
Hyperlipaemia without clinical atherosclerosis	♂	9	$\begin{cases} y_x = 0.103x - 0.106 \\ y = 0.56x - 1.37 \end{cases}$	0.00916 0.2042	0.0672 1.036	0.9 0.68
	♀	6	$\begin{cases} y = 0.020x + 0.092 \\ y = 0.28x - 0.13 \end{cases}$	0.01412 0.1020	0.0344 0.441	0.06 0.75
Hyperlipaemia with clinical atherosclerosis	♂	9	$\begin{cases} y = 0.008x + 0.138 \\ y = 0.08x + 0.84 \end{cases}$	0.00638 0.0688	0.0366 0.781	0.01 0.29
	♀	6	$\begin{cases} y_x = 0.064x - 0.040 \\ y_x = 0.37x - 0.47 \end{cases}$	0.00358 0.2013	0.0787 0.740	0.90 0.60
Normocholesterolaemic normo glyceridaemic atherosclerotic	♂	8	$\begin{cases} y = 0.006x + 0.092 \\ y = 0.75x - 1.37 \end{cases}$	0.00828 0.1600	0.0090 0.507	0.81 0.83

fasting-serum turbidity (about twice as high) than the group with clinical atherosclerosis. This suggests that the former a chylomicron phase might have a higher glyceride-glycerol content. Hence the regression of the glyceride-glycerol level of the supernatant ( $y$ ) upon that of the corresponding fasting whole serum ( $x$ ) was studied in the combined groups with (1) and without (2) clinical atherosclerosis. The respective equations were

$$\begin{aligned} (1) \quad y &= 0.61x + 0.67 \quad n = 46 \\ s_b &= 0.0473 \quad s_{b \cdot x} = 0.526 \quad r = 0.89 \\ (2) \quad y &= 0.61x + 0.72 \quad n = 43 \\ s_b &= 0.0466 \quad s_{b \cdot x} = 0.588 \quad r = 0.88 \end{aligned}$$

These two regression lines do not deviate significantly in either slope or level. Hence no differences was demonstrated between the groups with and without clinical atherosclerosis in either the glyceride-glycerol content of the chylomicron phase of fasting serum or

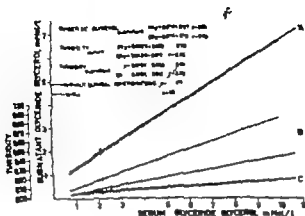


Fig. 8. Glyceride-glycerol as substrate of fasting serum, and turbidity of serum and substrate related to glyceride-glycerol of serum. A comparison between hypercholesterolemia and hyperlipidemia without and with clinical thrombocytosis.

the ratio of subnatant turbidity to whole fasting-serum glyceride-glycerol level. The fact then remains that these two groups differed with respect to the turbidity of the chylomieron phase of fasting serum (cf Fig 9).

Fasting serum turbidity and glyceride-glycerol were determined in a large number of subjects either as part of general medical check-ups or in conjunction with special examinations of patients with myocardial infarction blood donors etc. These laboratory findings were supplemented with the fasting values observed in the present series of fat tolerance tests. In order to estimate the screening value of serum turbidity the data were classified in order of increasing turbidity. The results have been graphed in Fig 10 which is based on some 900 estimations. In each turbidity class, comprising approximately 60 estimations, the glyceride-glycerol values show con-

siderable spread and a skew distribution. For example, in turbidity class 0 as the glyceride-glycerol values range from 0.4 to 1.4 mmol/l and the mean is of the order of 1 mmol/l. The corresponding figures for turbidity class 0.1 are 0.4 to 3.4 and 1.4 mmol/l.

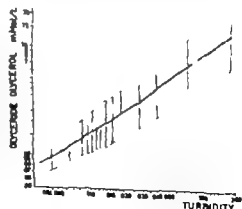


Fig. 10. Relation between glyceride-glycerol and turbidity in fasting serum. Broken lines represent 93% range.

TABLE 23 Regression of turbidity ( $y$ ) upon glyceride-glycerol ( $x$ ) before ( $S_0$ ) and 4 hours later ( $S_4$ ) the fast meal

Group	Sex	Regression equation $\left\{ \begin{matrix} y \\ y_1 \end{matrix} \right.$	$r$	$s_y$	$s_x$
Controls	♂ 38	$\begin{cases} y = 0.019x + 0.072 \\ y = 0.22x + 0.063 \end{cases}$	$\begin{matrix} 0.00346 \\ 0.0231 \end{matrix}$	$\begin{matrix} 0.0168 \\ 0.164 \end{matrix}$	$\begin{matrix} 0.18 \\ 0.74 \end{matrix}$
	♀ 31	$\begin{cases} y = 0.009x + 0.064 \\ y = 0.21x + 0.081 \end{cases}$	$\begin{matrix} 0.00530 \\ 0.0748 \end{matrix}$	$\begin{matrix} 0.0358 \\ 0.254 \end{matrix}$	$\begin{matrix} 0.09 \\ 0.43 \end{matrix}$
Hypercholesterolaemias without clinical atherosclerosis	♂ 8	$\begin{cases} y = 0.003x + 0.133 \\ y = 0.40x - 0.13 \end{cases}$	$\begin{matrix} 0.03485 \\ 0.1168 \end{matrix}$	$\begin{matrix} 0.0598 \\ 0.260 \end{matrix}$	$\begin{matrix} -0.03 \\ 0.77 \end{matrix}$
	♀ 22	$\begin{cases} y = 0.0004x + 0.080 \\ y = 0.58x - 0.69 \end{cases}$	$\begin{matrix} 0.01283 \\ 0.0854 \end{matrix}$	$\begin{matrix} 0.0247 \\ 0.237 \end{matrix}$	$\begin{matrix} 0.04 \\ 0.81 \end{matrix}$
Hypercholesterolaemias with clinical atherosclerosis	♂ 18	$\begin{cases} y = 0.015x + 0.062 \\ y = 0.18x + 0.13 \end{cases}$	$\begin{matrix} 0.00301 \\ 0.0444 \end{matrix}$	$\begin{matrix} 0.0537 \\ 0.197 \end{matrix}$	$\begin{matrix} 0.48 \\ 0.44 \end{matrix}$
	♀ 10	$\begin{cases} y = 0.021x + 0.019 \\ y = 0.12x + 0.18 \end{cases}$	$\begin{matrix} 0.00370 \\ 0.0620 \end{matrix}$	$\begin{matrix} 0.0108 \\ 0.187 \end{matrix}$	$\begin{matrix} 0.78 \\ 0.51 \end{matrix}$
Hyperlipaemias without clinical atherosclerosis	♂ 9	$\begin{cases} y = 0.103x - 0.106 \\ y = 0.56x - 1.37 \end{cases}$	$\begin{matrix} 0.00918 \\ 0.2042 \end{matrix}$	$\begin{matrix} 0.0672 \\ 1.058 \end{matrix}$	$\begin{matrix} 0.9 \\ 0.68 \end{matrix}$
	♀ 6	$\begin{cases} y = 0.020x + 0.093 \\ y = 0.28x - 0.13 \end{cases}$	$\begin{matrix} 0.01412 \\ 0.1020 \end{matrix}$	$\begin{matrix} 0.0344 \\ 0.441 \end{matrix}$	$\begin{matrix} 0.06 \\ 0.73 \end{matrix}$
Hyperlipaemias with clinical atherosclerosis	♂ 8	$\begin{cases} y = 0.008x + 0.128 \\ y = 0.08x + 0.84 \end{cases}$	$\begin{matrix} 0.00638 \\ 0.0888 \end{matrix}$	$\begin{matrix} 0.0366 \\ 0.781 \end{matrix}$	$\begin{matrix} 0.04 \\ 0.29 \end{matrix}$
	♀ 6	$\begin{cases} y = 0.061x - 0.040 \\ y = 0.37x - 0.47 \end{cases}$	$\begin{matrix} 0.00354 \\ 0.2013 \end{matrix}$	$\begin{matrix} 0.0267 \\ 0.740 \end{matrix}$	$\begin{matrix} 0.90 \\ 0.60 \end{matrix}$
Normocholesterolaemia, normo glyceridaemia atherosclerotic	♂ 8	$\begin{cases} y = 0.006x + 0.092 \\ y = 0.75x - 1.37 \end{cases}$	$\begin{matrix} 0.00628 \\ 0.1600 \end{matrix}$	$\begin{matrix} 0.0090 \\ 0.507 \end{matrix}$	$\begin{matrix} 0.61 \\ 1.25 \end{matrix}$

fasting-serum turbidity (about twice as high) than the group with clinical atherosclerosis. This suggests that the former a chylomicron phase might have a higher glyceride-glycerol content. Hence the regression of the glyceride-glycerol level of the subnatant ( $y$ ) upon that of the corresponding fasting whole serum ( $x$ ) was studied in the combined groups with (1) and without (2) clinical atherosclerosis. The respective equations were

$$\begin{aligned} (1) \quad y &= 0.61x + 0.67 \quad n = 45 \\ s_y &= 0.0473 \quad s_x = 0.628 \quad r = 0.89 \\ (2) \quad y &= 0.81x + 0.72 \quad n = 43 \\ s_y &= 0.0486 \quad s_x = 0.585 \quad r = 0.88 \end{aligned}$$

These two regression lines do not deviate significantly in either slope or level. Hence no differences was demonstrated between the groups with and without clinical atherosclerosis in either the glyceride-glycerol content of the chylomicron phase of fasting serum or

TABLE 4—Regression of serum glyceride-glycerol (y) upon subnatant glyceride-glycerol (x) 4 hours after the fast meal

Group	Sex	Regression equation			
Controls	♂ 28	$y = 0.85x + 0.39$	0.056	0.740	0.81
	31	$y = 0.84x - 0.01$	0.041	0.150	0.96
Hypercholesterolaemias without clinical atherosclerosis	♀ 8	$y = 0.70x + 0.19$	0.066	0.120	0.94
	12	$y = 0.80x + 0.24$	0.038	0.178	0.93
Hypercholesterolaemias with clinical atherosclerosis	♂ 18	$y = 0.51x + 0.77$	0.050	0.250	0.90
	10	$y = 0.78x - 0.37$	0.044	0.134	0.93
Hyperlipaemias without clinical atherosclerosis	♂ 9	$y = 0.31 + 3.02$	0.108	0.543	0.70
	6	$y = 0.89x + 0.03$	0.134	0.540	0.94
Hyperlipaemias with clinical atherosclerosis	♂ 9	$y = 0.33x + 1.3$	0.090	0.617	0.81
	6	$y = 0.75x - 0.60$	0.104	0.573	0.89
Normocholesterolaemic normo-lipidaemic hypercholesterolaemic	♂ 8	$y = 0.4x - 0.24$	0.117	0.370	0.81

0.28 mMol/l at whole serum glyceride-glycerol level of 0.2 and 1.76 mMol/l. In the latter the corresponding values were 1.28 and 0.8 mMol/l and 4.08 and 4.18 mMol/l respectively.

The regression of the glyceride-glycerol of the subnatant upon that of whole 4-hour serum for males and females in each group will be found in Table 4. The line of regression for men differs significantly in slope from that for women in each group except among hypercholesterolaemias without clinical atherosclerosis. But no difference appeared between the combined groups with and without clinical atherosclerosis. When the entire series was classified by sex only the regressions of the glyceride-glycerol of the subnatant upon that of whole serum were as shown in Fig. 11. Among females there was no significant difference between the controls; hypercholesterolaemias

and hyperlipaemias, so in Fig. 6 these are represented by a single line having the equation

$$y = 0.77x + 0.13 \quad n = 8 \\ s = 0.028 \quad s_{\text{y}} = 0.328 \quad r = 0.98$$

For the male groups the lines representing the regression of the glyceride-glycerol level of the subnatant upon that of whole serum disclosed that the line for the hypercholesterolaemias deviated significantly in level and that for the hyperlipaemias in slope from the other lines (cf. Fig. 11). Accordingly neither classification by the glyceride-glycerol of whole 4-hour serum nor analysis of the regression of the glyceride-glycerol of the subnatant upon that of whole 4-hour serum disclosed any significant difference between the groups with and without clinical atherosclerosis. But there was a significant difference between men and women.

Conversion of the glyceride-glycerol values into logarithms yields a more normal distribution within turbidity classes. The means and ranges shown in the curve were calculated from such logarithms. The curve was constructed after twice calculating successive means and ranges and reconverting these into the corresponding glyceride-glycerol values. As appears from Fig 10 the turbidity and glyceride-glycerol level of fasting serum are to some extent associated but the great spread severely limits the screening value of the turbidity measurements. At serum turbidity values not exceeding 0.08 none of the 140 glyceride-glycerol values was over 3 mMol/l and as many as 50 per cent were below 1 mMol/l. The chance that a single turbidity measurement of 0.14 will be accompanied by a glyceride-glycerol level exceeding 2 mMol/l is about 50 per cent and it will not exceed 5 mMol/l

Glyceride-glycerol of  
whole serum and its subnatant  
4 hours after the fat meal

Mean glyceride-glycerol of whole serum and its subnatant as well as their difference — the chylomicron phase

— 4 hours after the fat meal are specified for each group in Table 7 page 23. As appears from the table the mean glyceride-glycerol of whole 4 hour serum for males exceeded that for females in each group. Moreover the glyceride-glycerol of the chylomicron phase was higher when the glyceride-glycerol of whole serum was high than when it was low. To compare the glyceride-glycerol of the chylomicron phase in males and females the series was classified according to the glyceride-glycerol of whole serum and the mean glyceride-glycerol of the subnatant in per cent of the mean glyceride-glycerol of whole serum was calculated for males and females in each such class up to whole 4 hour serum glyceride-glycerol of 8 mMol/l. See Table 20. The subnatant was relatively higher for women than for men in each group. In other words the males had a larger chylomicron phase than the females.

In the combined groups with whole serum glyceride-glycerol of 0–3 and of 3–8 mMol/l the glyceride-glycerol of the chylomicron phases for men and women differed significantly ( $\chi^2 > 3.84$ ). In the first group the mean chylomicron phases were 0.44 and

TABLE 20 Glyceride-glycerol & subnatant in per cent of glyceride-glycerol serum 4 h after (4)

Sex	$\Delta$ -classes, mMol/l					b
	0–1	1–3	3–5	5–8	8–	
Men	88	79	7	69	69	a
n =	5	19	20	1	7	3
Women	87	82	83	80	83	b
n =	5	24	23	7	1	5

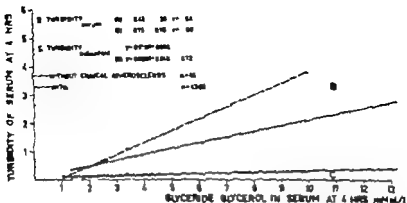


Fig. 12. Glyceride-glycerol in subsant of serum 4 hour after the fat meal, and turbidity of serum and subsant, related to glyceride-glycerol of serum. A comparison for the hypercholesterolaemia and hyperlipaemia without and with clinical atherosclerosis.

significant regression of turbidity upon glyceride-glycerol of whole serum existed. In this respect no difference could be demonstrated between men and women. Groups with clinical atherosclerosis differed from those without, however, as much as the turbidity relative to the glyceride-glycerol level was lower in the former than in the latter. The importance of this definite difference may be somewhat questioned by the different average age between groups with and without clinical atherosclerosis.

Glyceride-glycerol of whole serum and its subsant 7 hours after the fat meal

As appears from Table 7, page 23, the glyceride-glycerol of whole 7-hour serum and of its subsant differed significantly for the male controls, the male controls hypercholesterolaemic and hyperlipaemic. Since analysis de-

monstrated no differences between men and women or between groups with and without clinical atherosclerosis with respect to the regression of the glyceride-glycerol of the subsant upon that of whole 7-hour serum, this relationship was studied in (1) the controls regardless of sex (2) a group consisting of all hypercholesterolaemic and normocholesterolaemic normoglycaemic atherosclerosis and (3) a group comprising all hyperlipaemics. The lines for the regression of the glyceride-glycerol level of the subsant ( $y$ ) upon that of whole 7-hour serum ( $x$ ) in these groups are shown in Fig. 12 and have the following equations:

- (1)  $y = 0.075x + 0.002$   $n = 10$   
 $s = 0.048$   $s_{xx} = 0.149$   $r = 0.98$
- (2)  $y = 0.085x + 0.002$   $n = 10$   
 $s = 0.051$   $s_{xx} = 0.203$   $r = 0.98$
- (3)  $y = 0.142x + 0.27$   $n = 10$   
 $s = 0.128$   $s_{xx} = 0.475$   $r = 0.88$



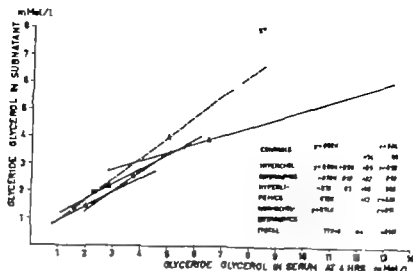


Fig. 11 Relation between glyceride-glycerol in serum 4 hours after the fat meal and in subnatant (25 000 g) in men (—) and women (---).

### Turbidity and glyceride-glycerol of whole 4-hour serum

The regressions of the turbidity upon the glyceride-glycerol of whole 4 hour serum for males and for females in the aforementioned groups regardless of the presence of clinical atherosclerosis showed no statistically significant differences. Therefore men and women were combined and the regression of turbidity ( $y$ ) upon the glyceride-glycerol of whole 4 hour serum became

$$y = 0.30x - 0.09 \quad n = 100 \\ s_b = 0.021 \quad s_y = 0.311 \quad r = 0.4$$

The constant in this equation differs significantly from zero.

However the regression of turbidity upon glyceride-glycerol of whole 4 hour serum showed a significant difference between those with and those without clinical atherosclerosis among the hypercholesterolaemics as well as among the hyperlipaemics. The regressions of

the turbidity ( $y$ ) upon the glyceride-glycerol ( $x$ ) of whole 4 hour serum in the combined hypercholesterolaemic and hyperlipaemic groups without (1) and with (2) clinical atherosclerosis (Fig. 12) have the equations

$$(1) \quad y = 0.42x - 0.39 \quad n = 45 \\ s_b = 0.040 \quad s_y = 0.381 \quad r = 0.84 \\ (2) \quad y = 0.10x + 0.15 \quad n = 47 \\ s_b = 0.040 \quad s_y = 0.38 \quad r = 0.60$$

The slopes of these regression lines deviate significantly<sup>1)</sup>

This difference between the groups with and without clinical atherosclerosis has no corollary in either the subnatant or the chylomicron phase of 4 hour serum.

In sum then the following can be said about the association between the turbidity and glyceride-glycerol of serum 4 hours after the fat meal.

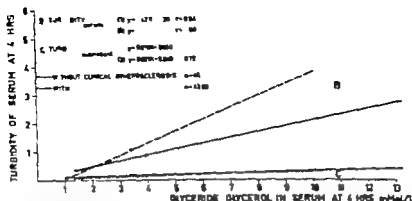


Fig 12. Glyceride-glycerol in subnatant of serum 4 hours after the fat meal, and turbidity of serum and subnatant, related to glyceride-glycerol of serum. A comparison between hypercholesterolaemics and hyperlipaemics without and with clinical atherosclerosis.

significant regression of turbidity upon glyceride-glycerol of whole serum existed. In this respect no difference could be demonstrated between men and women. Groups with clinical atherosclerosis differed from those without however in as much as the turbidity relative to the glyceride-glycerol level was lower in the former than in the latter. The importance of this definite difference may be somewhat questioned by the different average age between groups with and without clinical atherosclerosis.

Glyceride-glycerol of whole serum  
and its subnatant 7 hours  
after the fat meal

As appears from Table 7 page 23 the glyceride-glycerol of whole 7-hour serum and of its subnatant differed significantly for the male controls, female controls, hypercholesterolaemics and hyperlipaemics. Since analysis de-

monstrated no differences between men and women or between groups with and without clinical atherosclerosis with respect to the regression of the glyceride-glycerol of the subnatant upon that of whole 7 hour serum this relationship was studied in (1) the controls regardless of sex, (2) a group consisting of all hypercholesterolaemics and normocholesterolaemics normoglycaemic atherosclerotics and (3) a group comprising all hyperlipaemics. The lines for the regression of the glyceride-glycerol level of the subnatant ( $y$ ) upon that of whole 7 hour serum ( $x$ ) in these groups are shown in Fig 13 and have the following equations:

- (1)  $y = 0.87x + 0.003$   $n = 1$   
 $s_y = 0.046$   $s_x = 0.149$   $r = 0.92$
- (2)  $y = 0.90x + 0.007$   $n = 1$   
 $s_y = 0.034$   $s_x = 0.08$   $r = 0.98$
- (3)  $y = 0.75x + 0.27$   $n = 10$   
 $s_y = 0.128$   $s_x = 0.473$   $r = 0.88$

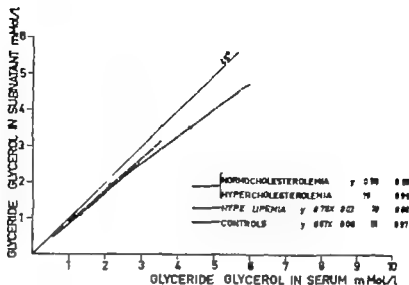


Fig 13 Relation between glyceride glycerol in serum and its subnatant 2 (25 000 g) hours after the fat meal.

The constant does not differ from zero in any of these three equations nor are their regression coefficients significantly unequal and only that for the controls deviates significantly from unity

When the subjects were classified according to whole 7 hour serum glyceride-glycerol levels of 0-2 ( $n = 32$ ) 2-4 ( $n = 13$ ) and 4-8 ( $n = 7$ ) mMol/l the mean subnatant glyceride glycerol levels in per cent of the corresponding whole serum means were found to be 80 87 and 81 per cent respectively

#### Turbidity and glyceride-glycerol of whole 7 hour serum

The regression of turbidity ( $y$ ) upon glyceride glycerol level ( $x$ ) of whole 7 hour serum for (1) controls (2) hypercholesterolaemics and hyperlipaemics without clinical atherosclerosis and (3)

all subjects with clinical atherosclerosis has the following equations

- (1)  $y = 0.10x + 0.08$   $n = 21$   
 $s_b = 0.026$   $s_y = 0.084$   $r = 0.88$
- (2)  $y = 0.12x + 0.13$   $n = 12$   
 $s_b = 0.04$   $s_y = 0.70$   $r = 0.80$
- (3)  $y = 0.12x + 0.05$   $n = 10$   
 $s_b = 0.00$   $s_y = 0.234$   $r = 0.56$

These regression lines do not differ significantly in either slope or level

The regressions of turbidity upon glyceride-glycerol level of serum immediately before as well as 4 and 7 hours after the fat meal are illustrated in Fig 14 The slopes of these regression lines differ significantly But the corresponding subnatants exhibited no difference in this respect Hence the difference was to be found in the chylomicron phase where the following regressions of turbidity ( $y$ ) upon glyceride-glycerol ( $x$ ) were obtained for

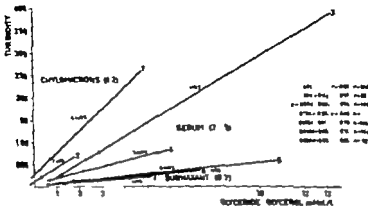


Fig 14. Relation between turbidity and glyceride-glycerol in serum, in it subnormal and chylomicron phase before 4 and 7 hours after the fat meal.

the chylomicron phase of (1) 4-hour serum and ( ) 7 hour serum

- (1)  $y = 0.462x + 0.37$   $n = 166$   
 $s_y = 0.044$   $s_{yx} = 0.568$   $r = 0.96$
- (2)  $y = 0.302x + 0.14$   $n = 52$ ,  
 $s_y = 0.076$   $s_{yx} = 0.319$   $r = 0.47$

Both the slopes and the levels of these lines deviate significantly ) Accordingly at equal glyceride-glycerol levels of the chylomicron phase 4 hour serum seems to have greater chylomicron turbidity than 7-hour serum in these groups.

## CHAPTER IX

### EFFECT OF DIETETIC CHANGES ON THE FAT TOLERANCE TEST

#### Definition of diets

*Ordinary diet* in the present context means the patient's ordinary diet or the regular hospital diet both of which contain predominantly fats of the saturated type

*Low-saturated fats diet* implies that the patient was asked to refrain from eating ordinary milk cream butter cheese eggs fat meats etc Most subjects on this diet lost a few kilos in body weight suggesting that it provided a somewhat lowered calorie intake

*High poly unsaturated fats diet* The low saturated fats diet with a daily supplement of 50 ml of oil with a minimum poly unsaturated fats content of 55 per cent

#### Technique for repeated fat tolerance tests

The fat tolerance test on ordinary diet was a rule made while the patient was admitted to hospital for a general check up Upon discharge the patient was put on a low-saturated fats diet for six weeks whereupon the fat tolerance test was repeated in the out patient department Then the patient was put on a high poly unsaturated fats diet for another six weeks and the fat tolerance test was repeated once more in the out patient department.

#### Fat tolerance tests on ordinary diet and on a high poly unsaturated fats diet

The alterations in the fat tolerance test resulting after a change from ordinary diet to a high poly unsaturated fats diet was studied in 32 subjects 16 hypercholesterolaemics and 16 by periphaemics. Mean cholesterol phospholipid glyceride-glycerol and turbidity values for fasting and 4 hour sera at the repeated fat tolerance tests are presented in Table 28 The table reveals that the means of all lipid factors in serum immediately before the fat meal were significantly reduced when the patients fat intake was changed from saturated to poly unsaturated fats

The regression of the serum glyceride-glycerol immediately before ( $x$ ) on that 4 hours after ( $y$ ) the fat meal when the same patient's fat tolerance test on ordinary diet (1) was repeated on a high poly unsaturated fats diet (2) has the equations

$$(1) \quad y = 1.30x + 0.67 \quad n = 32 \\ s_b = 0.089 \quad s_{y,x} = 1.05 \quad r = 0.93$$

$$(2) \quad y = 1.28x + 0.44 \quad n = 32 \\ s_b = 0.080 \quad s_y = 0.58 \quad r = 0.92$$

Neither the coefficients of regression nor the constants were significantly unequal The differences between the means of  $x$  (3.08 and 1.86 mmol/l re

TABLE 21. Fat tolerance test on 22 subjects on ordinary diet and repeated on diet restricted in saturated fat and polyunsaturated fat added

	Cholesterol mg per cent		Phospholipids mg per cent		Glyceride-glycerol mMol/l		Turbidity	
	before	4 hrs	before	4 hrs	before	4 hrs	before	4 hrs
Ordinary diet	360.1	364.0	312.1	340.5	3.09	4.61	0.16	1.18
Diet rich pol unsaturated fat	310.2	304.0	310.8	313.2	1.84	2.43	0.11	0.63
Difference ( $\bar{d}$ )	49.9	60.0	32.6	48.5	1.19	1.81	0.05	0.55
	12.4	9.7	11.1	10.8	0.59	0.44	0.02	0.19
	4.05	8.70	2.91	4.37	4.00	4.14	2.80	2.96
Decrease in per cent	14	18	10	11	29	27	23	4

pectively) and the means of  $y$  (4.64 and 2.82 mMol/l respectively) are significant

Unlike the case of duplicate fat tolerance tests (cf Table 14 p 35) the fasting serum exhibited no significant association between either cholesterol or phospholipids or turbidity on the one hand and glyceride-glycerol on the other when the fat tolerance test was repeated after substitution of saturated fats by poly unsaturated fats ( $\chi^2$  test). This phenomenon might conceivably be accounted for by the possibility that such a change in the patient's diet occasionally is attended by dissociation of the cholesterol phospholipid and glyceride-glycerol changes. For example at the repeated fat tolerance tests a man of 51 had fasting sera with cholesterol levels of 307 and 293 mg %, phospholipid levels of 323 and 301 mg %, and glyceride-glycerol levels of 8.58 and 3.88 mMol/l. Similarly on the two occasions the fasting serum of a woman of 53 showed cholesterol

values of 441 and 303 mg %, phospholipid values of 422 and 307 mg %, glyceride-glycerol values of 2.82 and 2.0 mMol/l and turbidity values of 0.13 and 0.07.

In 15 cases (8 hypercholesterolaemics and 7 hyperlipaemics) similar comparisons were made between the fasting 4 hour and 7 hour values. As appears from Table 20 the greatest differences between fat tolerance tests repeated on different diets appeared 7 hours after the fat meal while the fasting values exhibited the smallest differences. Whereas the turbidity values in either fasting or 4-hour or 7-hour sera showed no significant changes, significant decreases were observed in all 4 hour and 7 hour values for cholesterol, phospholipids and glyceride-glycerol.

The regression of the glyceride-glycerol level of 7-hour serum ( $y$ ) upon that of fasting serum ( $x$ ) when the fat tolerance test was repeated after a change from ordinary diet (1) to a

## CHAPTER IX

### EFFECT OF DIETETIC CHANGES ON THE FAT TOLERANCE TEST

#### Definition of diets

*Ordinary diet* in the present context means the patient's ordinary diet or the regular hospital diet both of which contain predominantly fats of the saturated type

*Low-saturated fats diet* implies that the patient was asked to refrain from eating ordinary milk, cream butter cheese, eggs fat meats, etc. Most subjects on this diet lost a few kilos in body weight suggesting that it provided a somewhat lowered calorie intake.

*High poly-unsaturated fats diet* The low saturated fats diet with a daily supplement of 30 ml of oil with a minimum poly unsaturated fats content of 55 per cent

#### Technique for repeated fat tolerance tests

The fat tolerance test on ordinary diet was a rule made while the patient was admitted to hospital for a general check up. Upon discharge the patient was put on a low-saturated fats diet for six weeks whereupon the fat tolerance test was repeated in the out patient department. Then the patient was put on a high poly unsaturated fats diet for another six weeks and the fat tolerance test was repeated once more in the out patient department

#### Fat tolerance tests on ordinary diet and on a high poly unsaturated fats diet

The alterations in the fat tolerance test resulting after a change from ordinary diet to a high poly unsaturated fats diet was studied in 32 subjects 16 hypercholesterolaemic and 16 hypolipaeemics. Mean cholesterol phospholipid glyceride-glycerol and turbidity values for fasting and 4-hour sera at the repeated fat tolerance tests are presented in Table 78. The table reveals that the means of all lipid factors in serum immediately before the fat meal were significantly reduced when the patient's fat intake was changed from saturated to poly unsaturated fats.

The regression of the serum glyceride-glycerol immediately before ( $x$ ) on that 4 hours after ( $y$ ) the fat meal when the same patient's fat tolerance test on ordinary diet (1) was repeated on a high poly unsaturated fats diet (2) has the equations

$$(1) \quad y = 1.30x + 0.67 \quad n = 32 \\ s_y = 0.080 \quad s_{y,x} = 1.05 \quad r = 0.99$$

$$(2) \quad y = 1.28x + 0.44 \quad n = 32 \\ s_y = 0.080 \quad s_{y,x} = 0.56 \quad r = 0.92$$

Neither the coefficients of regression nor the constants were significantly unequal. The difference between the means of  $x$  (3.08 and 1.46 mMol/l re

TABLE 30. Cholesterol and phospholipid change in subjects showing decrease in fasting serum of at least 10 mg% on diet restricted in saturated fat and poly-unsaturated fat added

	Cholesterol mg%			Phospholipid mg%		
	N	PUFA	N-PUFA	N	PUFA	N-PUFA
<i>S</i>	340 - 1	332	- 8	337 - 19	346	- 1
<i>A</i> Diet 9	- 11	- 3.5	- 4.6	+ 6.5	+ 14.4	- 2.1
4 hr	31.0	7.3	31.1	33.3	8.5	39.5
diff sign $\neq 0$	no	yes	no	yes	yes	no
<i>B</i>	345 - 11	346	9	367 - 9	373	6
<i>B</i> Diet 8	8.0	- 2.9	- 3.1	+ 3.1	+ 13.6	- 14.1
$\pi$	30.9	32.9	45.1	33.4	1.4	40.4
diff sign $\neq 0$	no	no	no	no	no	no

N = Ordinary diet with saturated fat

PUFA = Diet restricted in saturated fat and poly-unsaturated fat added

difference in the spread of both the cholesterol and the phospholipid changes from immediately before to 4 hours after the fat meal. In both cases this spread was significantly narrower on the diet rich in poly unsaturated fats. This could be a manifestation of more stable metabolic conditions on the latter diet.

Corresponding studies for fasting and 7 hours values were made in a cholesterol group of 7 hypercholesterolaemics and 4 hyperlipaemics and a phospholipid group of 5 hypercholesterolaemics and 4 hyperlipaemics. The results are presented in Table 30 b. It discloses no differences between the serum cholesterol changes. Phospholipids were significantly higher in hour serum than in fasting serum on

ordinary diet but not on a high-poly unsaturated fats diet. No significant difference in the phospholipid change could be demonstrated. The spread of the cholesterol and phospholipid changes were smaller on the latter diet (significantly so for cholesterol only).

Fat tolerance tests on ordinary diet and on a low-saturated fats diet

Fat tolerance tests repeated after a change from ordinary diet to a low saturated-fats diet were compared in 13 cases, 7 hypercholesterolaemics and 6 hyperlipaemics. Both immediately before and 4 hours after the fat meal, the serum cholesterol, phospholipid, glyceride-glycerol and turbidity means



TABLE 20 Serum lipids before 4 and 7 hours after the fat meal in 8 hypercholesterolaemic and hyperlipaemic on ordinary diet (N) and on diets restricted in saturated fat and polyunsaturated fat added (PUFA)

	Cholesterol mg%				Phospholipid mg %				Glyceride-glycerol mMol/l				Turbidity			
	Diet		diff		Diet		diff		Diet		diff		Diet		diff	
	N	PUFA			N	PUFA			N	PUFA			N	PUFA		
0 hr	383	344	39.2	93	341	314	26.1	1.62	180	175	0.75	0.53	0.14	0.17	0.00	1.9
			±0.3				18.4				0.30				0.018	
4 hrs	387	322	65.7	5.16	371	328	42.3	2.03	280	173	1.07	2.23	0.88	0.1	0.115	0.3
			12.9				14.3				0.46				0.021	
7 hrs	385	318	70.9	6.01	380	320	61.0	3.83	286	204	1.52	5.30	0.93	0.47	0.462	1.89
			11.8				13.3				0.39				0.234	

= standard deviation

high polyunsaturated fats diet (2) satisfied the equations

- (1)  $y = 1.31x + 0.54$   $n = 15$   
 $s_y = 0.1$   $s_x = 1.336$   $r = 0.75$   
 (2)  $y = 1.6x + 0.8$   $n = 15$   
 $s_y = 0.267$   $s_x = 0.8$   $r = 0.77$

The lines corresponding to these equations did not differ significantly in either slope or level. Corresponding mean serum glyceride-glycerol level before and 7 hours after the fat meal differed significantly. Similar regressions of the serum glyceride-glycerol level 7 hours after (x) upon that 4 hours after (y) when the fat intake was changed from saturated (1) to polyunsaturated fats (2) were compared. The following equations were obtained:

- (1)  $y = 1.01x - 0.22$   $n = 15$   
 $s_y = 0.1$   $s_x = 0.897$   $r = 0.90$   
 (2)  $y = 0.84x + 0.05$   $n = 15$   
 $s_y = 0.0$   $s_x = 0.288$   $r = 0.95$

Nor did these lines deviate in either slope or level.

As a corollary to what was done with the duplicate fat tolerance tests (Chapter 1, page 32) these cases were studied in which the dietetic change caused the repeated fat tolerance test to show a fasting serum cholesterol decrease ( $n = 21$ ) and/or phospholipid decrease ( $n = 19$ ) exceeding 20 mg. The cholesterol group comprised 1 hypercholesterolaemics and 9 hyperlipaemics and the phospholipid group 9 hypercholesterolaemics and 10 hyperlipaemics. The results are presented in Table 30a. The table reveals that such significant differences in the cholesterol and phospholipid changes from immediately before to 4 hours after the fat meal as could be demonstrated at the duplicate fat tolerance test did not appear when the fat tolerance test on ordinary diet was repeated on a high polyunsaturated fats diet. On the other hand there emerged a significant

TABLE 31 Fat tolerance test on 15 subjects on ordinary diet and on diet restricted in saturated fat

	Cholesterol mg <sup>100</sup>		Phospholipid mg <sup>100</sup>		Glyceride-gly mMol/l		Turbid y	
	before	4 hrs	before	4 hrs	before	4 hrs	before	4 hrs
Ordinary diet	315	318	300	304	3.05	3.83	0.14	1.41
Restricted diet	294	302	294	312	2.52	2.75	0.11	0.70
Difference ( $\bar{x}$ )	21.7	15.4	11.9	14.5	0.63	1.24	0.030	0.742
	15.3	16.3	11.7	1.3	0.61	0.81	0.041	0.55
Mean of $\bar{x}$	1.37	0.97	0.81	0.83	1.50	1.24	1.3	3.03
Decrease in %	7	6	4	4	17	3	29	3

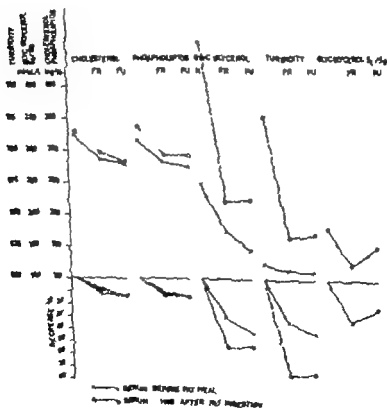


FIG. 15. Standard fat meal on ordinary diet (NY) diet restricted in saturated fat (FR) and FR diet + h poly-unsaturated fat added (PU).

were lower after restriction of saturated fats but the turbidity of 4 hour serum exhibited the only significant difference (Table 31)

The regression of the same subject's serum glyceride-glycerol level 4 hours after the fat meal ( $y$ ) upon that immediately before it ( $x$ ) fitted the following equations on ordinary diet (1) and on a low saturated fats diet (2)

$$(1) \quad \bar{y} = 0.05x + 2.13 \quad n = 13 \\ s_y = 0.105 \quad s_{y \cdot x} = 1.380 \quad r = 0.80$$

$$(2) \quad \bar{y}_x = 1.30x + 0.4 \quad n = 13 \\ s_y = 0.24 \quad s_{y \cdot x} = 1.368 \quad r = 0.78$$

These regression lines did not deviate significantly in either slope or level. Hence the association between the serum glyceride-glycerol level 4 hours after and immediately before the fat meal on ordinary diet seemed substantially similar to that on a low saturated fats diet. The regression line corresponding to equation (2) is a good approximation of the corresponding line for the larger series ( $n = 32$ ) on ordinary diet.

In only 6 subjects (3 hypercholesterolemics and 3 hyperlipaemias) was the fat tolerance test on ordinary diet repeated on a low-saturated fats diet then once more on a high poly unsaturated fats diet. Means for lipids and turbidity immediately before and 4 hours after the fat meal on the three diets are shown in Fig. 15 revealing that the greatest serum lipid reduction occurred on the diet with restricted saturated fats while the subsequent addition of poly unsaturated fats diet maintained the levels or reduced them slightly

more. Cholesterol and phospholipids exhibited the lowest percentage decreases (10–15%) while the glyceride-glycerol and turbidity of serum were reduced 30–40 per cent immediately before and a bit more 4 hours after the fat meal.

Summarizing the following can be stated:

A When fat tolerance tests on ordinary diet were repeated on a high poly unsaturated fats diet and the fasting serum lipids and turbidity decreased significantly the mean differences in lipids and turbidity between the 7 hour sera were larger and had smaller standard deviations than those for the fasting sera. This suggests that in this respect the fat tolerance test could be of some use.

B The cholesterol and phospholipid changes from immediately before to 4 hours after the fat meal were greater on ordinary diet than on a diet rich in poly unsaturated fats (not significant) and their spread is significantly smaller in the latter case.

C The largest reductions in serum lipids and turbidity are induced by a diet with restriction of saturated fats and addition to that diet of supplementary poly unsaturated fats maintains or slightly accentuates these reductions.

### Comment

The results of a fat tolerance test are highly dependent on the subject's diet during the period preceeding the test. Whereas isocaloric fat restriction increased the fat tolerance (POMER

## GENERAL DISCUSSION

For the last 50 years fat tolerance tests involving lipid estimations, chylomicron counts and turbidity measurements in serum have been performed in a variety of ways. Balanced test meals combining both carbohydrates and proteins with fat have been used very widely. Such meals would no doubt be preferable were it not for the fact that the serum triglycerides are influenced not only by carbohydrates but also in all probability by proteins. And the introduction of so many imponderables must make it exceedingly difficult to draw valid conclusions. A standard meal composed exclusively of fats would seem to be superior in yielding reproducible experiments.

(Cold double cream proved to be a well tolerated and readily standardizable meal. It might seem a good idea to adjust the amount of fat administered to the weight of the subject, but the present investigation did not demonstrate any association between the serum lipids neither immediately before nor 4 hours after a standard fat meal and body weight, stature or body surface area.

*Reproducibility.* The majority of previous workers have expressed the view that fat tolerance tests yield reasonably reproducible results. The methods

of choice has been to plot curves delineating the individual's lipid changes in serum at duplicate fat tolerance tests and these have been accepted as similar. On the other hand, no previous author seems to have attempted to estimate the magnitude of the variations. The present investigation revealed that the serum lipids and the turbidity before and 4 hours after the fat meal are associated. In analysis of the changes in serum induced by a fat meal the first step was to estimate the range of normal variation of the serum lipids and turbidity before the fat meal in three groups of patients: hypercholesterolaemia, hyperlipaemia and normocholesterolaemia, normoglyceridaemia. The standard deviation in per cent of the mean was 10–11 per cent for cholesterol, 1–20 per cent for phospholipids, 21–73 per cent for glyceride-glycerol, and 64–235 per cent for turbidity.

Considering that the lipids and turbidity vary widely even in fasting serum, one might well ponder whether there is any point in talking about such a thing as reproducibility of a fat tolerance test. — When subjects with a large glyceride-glycerol difference in fasting serum were selected, it turned out that the ratio of the serum glyceride-glycerol level 4 hours after

RANZE BRINFIELD & CHESSIN 1954  
HAVEL 1957 BRONTE-STEWART &  
BLACKBURN 1958) an increase of the  
dietary fat intake from 40 to 54 per  
cent for 3 weeks did not affect the fat  
tolerance (HORLICK, 1957)

Administration of poly unsaturated  
fats to subjects with ischaemic heart  
disease and high turbidity curves caused  
their fat tolerance to become more like  
that of the controls (BRONTE-STEWART  
& BLACKBURN 1958)

The writer's observations bear out  
previous findings. In the present in-  
vestigation isocaloric fat restriction was  
not attempted but merely restriction  
of saturated fats. This means that the  
relation between saturated and un-  
saturated fats was shifted in favour  
of unsaturated dietary fats. Hence the  
caloric intake was reduced as mani-  
fested by the fact that the patients  
lost some 2 kg body weight over the

period of 6 weeks they were on the  
low-saturated fats diet. When they  
subsequently received supplementary  
poly unsaturated fats in the diet their  
body weight generally returned to  
what it had been on ordinary diet.  
The increased fat tolerance persisted  
or was accentuated after addition to  
the low-saturated fats diet of supple-  
mentary poly unsaturated fats. As a  
measure of the fat tolerance the lipid  
values of 7 hour seemed superior.

The analysis of duplicate fat toler-  
ance tests (cf Chapter 5) disclosed that  
the test with the lower fasting value  
showed the greater cholesterol or phos-  
pholipid change 4 hours after the fat  
meal. Conversely, when the fat toler-  
ance test on ordinary diet was repeated  
on a high poly unsaturated fats diet  
the test with the lower fasting serum  
phospholipid level showed if anything  
the smaller phospholipid change.

## GENERAL DISCUSSION

For the last 50 years fat tolerance tests involving lipid estimations, chylomicron counts and turbidity measurements in serum have been performed in a variety of ways. Balanced test meals, combining both carbohydrates and proteins with fat, have been used very widely. Such meals would no doubt be preferable were it not for the fact that the serum triglycerides are influenced not only by carbohydrates but also in all probability by proteins. And the introduction of so many imponderables must make it exceedingly difficult to draw valid conclusions. A standard meal composed exclusively of fats would seem to be superior in yielding reproducible experiments.

Cold double cream proved to be a well tolerated and readily standardizable meal. It might seem a good idea to adjust the amount of fat administered to the weight of the subject, but the present investigation did not demonstrate any association between the serum lipids neither immediately before nor 4 hours after a standard fat meal and body weight, stature or body surface area.

*Reproducibility.* The majority of previous workers have expressed the view that fat tolerance tests yield reasonably reproducible results. The methods

of choice has been to plot curves delineating the individual's lipid changes in serum at duplicate fat tolerance tests and these have been accepted as similar. On the other hand, no previous author seems to have attempted to estimate the magnitude of the variations. The present investigation revealed that the serum lipids and the turbidity before and 4 hours after the fat meal are associated. In analysis of the changes in serum induced by a fat meal the first step was to estimate the range of normal variation of the serum lipids and turbidity before the fat meal in three groups of patients: hypercholesterolaemics, hyperlipaemics and normocholesterolaemic normoglyceridaemics. The standard deviation in per cent of the mean was 10–11 per cent for cholesterol, 12–20 per cent for phospholipids, 21–73 per cent for glyceride-glycerol, and 64–230 per cent for turbidity.

Considering that the lipids and turbidity vary widely even in fasting serum, one might well ponder whether there is any point in talking about such a thing as reproducibility of a fat tolerance test. — When subjects with a large glyceride-glycerol difference in fasting serum were selected, it turned out that the ratio of the serum glyceride-glycerol level 4 hours after

the fat meal to that immediately before it remained substantially constant from one occasion to another. In other words the relative glyceride glycerol rise on different occasions was of similar magnitude. If this fact is utilized for estimation of the "standard error" of a single fat tolerance test it is found that this is of the order of 20 per cent. And a "standard error" of this order requires that the groups of patients under study differ a good deal or that the groups are very large.

*The individual's response to the fat meal.* An individual's response to the fat meal can be studied by letting him undergo duplicate fat tolerance tests. With the aid of this method the present investigation disclosed that for one and the same individual on the same diet on both occasions the turbidity readings can serve as a screening test — the higher turbidity is usually accompanied by the higher glyceride glycerol level. Furthermore the higher turbidity value and the higher glyceride glycerol value of fasting serum are as a rule both accompanied by the corresponding higher value for serum 4 hours after the fat meal. It was demonstrated with respect to serum cholesterol as well as to serum phospholipids that the lower of the two fasting values undergoes the greatest change in response to the fat meal. The serum cholesterol decreases occasionally observed in response to the fat meal could be responsible for the absence of appreciable cholesterol changes in the majority of investiga-

tions — the present one included — concerned with groups of patients.

*Fat tolerance tests in different categories of subjects.* The controls constituted a highly important group in the present investigation in so far as it was the sole group with random lipid factors in serum. All the other subjects had been assigned to particular groups on the basis of the values of the lipid factors in the fasting serum. In the case of the control group it appeared that the glyceride glycerol level of serum both 4 hours and 7 hours after the fat meal was linearly related to the fasting value enabling estimation of either of the former values from the latter value. Thus in the controls the serum glyceride-glycerol level 4 hours and 7 hours after the fat meal was respectively 1.65 and 1.1 times the corresponding level of fasting serum. Only two groups of patients deviated from the first relationship. One of these groups comprised males with clinical atherosclerosis, normal serum cholesterol and serum glyceride-glycerol not exceeding 2 mMol/l. The distribution of this group about the regression line for the controls was on a significantly higher level.

Glyceride glycerol level of 4 hour serum equals 1.6 times the glyceride glycerol level of fasting serum up to about 4 mMol/l, a limit above which the 4 hour values are less than 1.65 times the fasting value.

In the control group the serum glyceride glycerol level 7 hours after the fat meal can be estimated at 1.17 times the fasting value. Yet the rela-

tion seems to hold only for glyceride-glycerol levels of fasting serum not exceeding  $\text{mMol/l}$ . Scattered glyceride-glycerol levels of fasting serum exceeding  $\text{mMol/l}$  were observed. 70 per cent of them lay above the line  $y = 1.1x$  and the frequency of extreme 7 hour values also seems to be higher at fasting values over  $\text{mMol/l}$ . What this means is that the serum glyceride-glycerol values 4 and 7 hours after the fat meal are strongly associated with the fasting value but that the nature or strength of the association changes at different levels of the fasting value. So far as these associations are concerned, atherosclerotics did not differ from non-atherosclerotics with the same fasting serum glyceride-glycerol.

Similarly the 7 hour glyceride-glycerol values are associated with the 4-hour values in a manner that depends upon the level of the 4-hour value. Thus 4 hour serum glyceride-glycerol values exceeding  $\text{mMol/l}$  tend to be accompanied by similar or higher 7 hour values whereas 4-hour values below  $\text{mMol/l}$  usually yield an approximately 20 per cent lower 7 hour value. Nor in this respect did atherosclerotics deviate from non-atherosclerotics. On the other hand hyperlipaemia and hypercholesterolaemia differed from the controls in having proportionately higher 7 hour values in relation to the 4-hour values. Assuming that the controls as well as the hypercholesterolaemia and hyperlipaemia attain the maximum on their serum glyceride-glycerol curve 4 hours after the fat meal, then the rate of

fat removal from the blood must be lower in the two latter groups than in the controls. The reason why so many investigators have reported differences between controls and atherosclerotics in this very removal phase after fat loading is presumably that selection of a group of atherosclerotics is tantamount to selection of subjects with hypercholesterolaemia and hyperlipaemia.

When the mean serum glyceride-glycerol levels immediately before as well as 4 and 7 hours after the fat meal are compared in different groups, allowance must be made for the interactions between the values. When such interactions are disregarded and groups are compared on the basis of respectively fasting 4-hour and 7-hour values, the result will be what most previous workers have done and which is correct to such interaction. Various comparisons of this type disclose that the serum glyceride-glycerol level is far superior to the turbidity as a criterion for distinguishing between groups of patients. Furthermore in this respect the glyceride-glycerol level of fasting serum is superior to the corresponding levels of either 4 hour serum or 7 hour serum. Accordingly it seems rather pointless to carry out the fat tolerance test.

*Serum and its subnanant* A further means of distinguishing between atherosclerotics and non atherosclerotics was probed. It constitutes the glyceride-glycerol distribution in the chylomicron phase and subnanant of serum. It was



demonstrated that the fasting serum of the controls had a chylomicron phase unlike the other groups in which the serum glyceride-glycerol level had to exceed 4 mMol/l before the chylomicron phase became significant. Conversely 4 hours after the fat meal all groups of subjects had a chylomicron phase regardless of the glyceride-glycerol level of whole serum and this chylomicron phase became proportionately larger the higher the whole serum glyceride glycerol. Here a difference became apparent between men and women — men have a higher proportion of their neutral fats in the form of chylomicrons than women. No corresponding difference was apparent 7 hours after the fat meal. No difference could be found between groups with and without clinical atherosclerosis in this respect.

An association exists between the turbidity and glyceride-glycerol level of serum and it appeared that the nature of this association in non atherosclerotics was different from that in atherosclerotics. This difference was significant for fasting serum. But glyceride-glycerol of the chylomicron phase of the same serum was equally large in these two groups (the regressions of the supernatant upon whole serum were practically identical as were the regressions of supernatant turbidity upon whole serum glyceride glycerol). The situation was similar for serum 4 hours after the fat meal but could not be demonstrated for serum 7 hours after the fat meal conceivably because 7 hour serum was obtained only from about one third of the number of

subjects supplying fasting serum and 4-hour serum. With respect to serum immediately before and 4 hours after the fat meal the conclusion may be drawn that the glyceride glycerol content of the chylomicron phase produces different degrees of whole serum turbidity in non atherosclerotics and atherosclerotics in other words the chylomicrons are qualitatively different. This suggests that there may exist chylomicrons which can be a factor in the development of atherosclerosis on account of their properties rather than their quantity.

The investigations described in the foregoing were designed to reveal whether atherosclerotics differ from non atherosclerotics in some way susceptible to detection by quantitative changes in serum glyceride-glycerol or turbidity in response to a fat meal. By and large the answer must be no. The two exceptions however may have definite importance. It should be restudied whether at every level of glyceride-glycerol a group with clinically proven atherosclerosis will have a less turbid serum than a group with no clinically detectable atherosclerosis. The second suggestive finding that the clinically atherosclerotic normocholesterolemic and normoglyceremic group of males had a significantly higher glyceride glycerol level at 4 hours than the average 1.55 times the fasting value is to some extent invalidated by the smallness of the group. This also calls for further studies.

*Fat tolerance tests on different diets*  
The fat tolerance of a few patients

was tested on a diet with predominantly saturated fats and then repeated on a diet with predominantly polyunsaturated fats. It appeared that the fasting glyceride-glycerol and turbidity values were approximately 40 per cent lower after the change and the response

to the fat meal similar in principle but on a lower level. On both occasions the equations for the regression of 4 hour glyceride-glycerol upon fasting glyceride-glycerol were similar and so were the equations for the regression of the 7 hour values upon the fasting values.

demonstrated that the fasting serum of the controls had a chylomicron phase unlike the other groups in which the serum glyceride-glycerol level had to exceed 4 mMol/l before the chylomicron phase became significant. Conversely 4 hours after the fat meal all groups of subjects had a chylomicron phase regardless of the glyceride-glycerol level of whole serum and this chylomicron phase became proportionately larger the higher the whole serum glyceride-glycerol. Here a difference became apparent between men and women — men have a higher proportion of their neutral fats in the form of chylomicrons than women. No corresponding difference was apparent 7 hours after the fat meal. No difference could be found between groups with and without clinical atherosclerosis in this respect.

An association exists between the turbidity and glyceride-glycerol level of serum, and it appeared that the nature of this association in non atherosclerotics was different from that in atherosclerotics. This difference was significant for fasting serum. But glyceride-glycerol of the chylomicron phase of the same serum was equally large in these two groups (the regressions of the supernatant upon whole serum were practically identical as were the regressions of supernatant turbidity upon whole serum glyceride-glycerol). The situation was similar for serum 4 hours after the fat meal but could not be demonstrated for serum 7 hours after the fat meal conceivably because 7-hour serum was obtained only from about one-third of the number of

subjects supplying fasting serum and 4-hour serum. With respect to serum immediately before and 4 hours after the fat meal the conclusion may be drawn that the glyceride-glycerol content of the chylomicron phase produces different degrees of whole serum turbidity in non atherosclerotics and atherosclerotics in other words the chylomicrons are qualitatively different. This suggests that there may exist chylomicrons which can be a factor in the development of atherosclerosis on account of their properties rather than their quantity.

The investigations described in the foregoing were designed to reveal whether atherosclerotics differ from non atherosclerotics in some way susceptible to detection by quantitative changes in serum glyceride-glycerol or turbidity in response to a fat meal. By and large the answer must be no. The two exceptions however may have definite importance. It should be restudied whether at every level of glyceride-glycerol a group with clinically proven atherosclerosis will have a less turbid serum than a group with no clinically detectable atherosclerosis. The second suggestive finding that the clinically atherosclerotic normocholesterolemic and normoglyceridemic group of males had a significantly higher glyceride-glycerol level at 4 hours than the average 1.63 times the fasting value is to some extent invalidated by the smallness of the group. This also calls for further studies.

*Fat tolerance tests on different diets*  
The fat tolerance of a few patients

female). Only \* of the other 8 groups differed significantly from the controls in this respect normocholesterolaemic normoglyceridaemic patients with clinical atherosclerosis having higher values and hyperlipaemic women without clinical atherosclerosis lower values than expected. The regression seems to be true up to glyceride-glycerol levels of 4 mMol/l for the other groups.

For the association between glyceride-glycerol in control serum before (x) and 7 hours after the fat meal (y) the following regression was found.  $y = 1.1x + 1.17$  is not  $= 1$  i.e. the glyceride-glycerol level in serum 7 hours after the fat meal did not differ significantly from the fasting level. The spread of the observations about this line was very great and the statistical analysis showed that the total material from the fat tolerance point of view was tolerably homogenous and the association for the whole material could be expressed by the regression  $y = 1.4x$ . The regression  $y = 1.17x$  is held to be true up to a serum level of mMol/l. No differences were shown between various groups without and with clinical atherosclerosis.

The association between glyceride-glycerol in serum 7 and 4 hours after the fat meal for levels below 4 mMol/l differed from that above this level. Below this level there was usually a decrease of 30 per cent and above it often an increase of glyceride-glycerol in serum between the 4th and 7th hour after the fat meal. No difference between groups without and

with clinical atherosclerosis was shown but hyperlipaemic and hypercholesterolaemic differed from the controls in having proportionately higher hour values in relation to 4-hour values.

No association was demonstrated between turbidity in serum before and 4 and 7 hours after the fat meal. Almost all comparisons between the various hypercholesterolaemic groups and the group with normocholesterolaemic normoglyceridaemic atherosclerosis demonstrated no differences from the corresponding control group either in fasting serum turbidity or in 4 and 7 hour values. Similar comparison revealed that glyceride-glycerol was superior for distinguishing clinical groups.

Glyceride-glycerol in the chylomicron phase did not differ in patients without and with clinical atherosclerosis either before or 4 and 7 hours after the fat meal. On the other hand there was a difference between men and women so that men had more chylomicron glyceride-glycerol than women 4 hours but not 7 hours after the fat meal. Controls had a small chylomicron phase in fasting but the other subjects showed no such phase below glyceride-glycerol levels of 4 mMol/l in fasting serum.

Subjects without clinical atherosclerosis differed from those with atherosclerosis as to the turbidity in relation to glyceride-glycerol in serum before and 4 hours after the fat meal, patients with clinical atherosclerosis having lower turbidity than those without. No difference was demonstrated be-

## GENERAL SUMMARY

The aim of this investigation was in the first place to study the value of the fat tolerance test in distinguishing subjects with and without atherosclerosis but also to compare the fat tolerance tests of an individual on ordinary diet with saturated fat and on a diet with polyunsaturated fat. The fat meal consisted of 200 ml 40% cream. Serum turbidity, glyceride-glycerol, cholesterol and phospholipids were determined before as well as 4 and 7 hours after the fat meal in a) controls (males and females), b) hypercholesterolaemias (male and females), c) hyperlipaemias (males and females) and d) normoglyceridaemic normocholesterolaemias with atherosclerosis (males only). Thus totally 11 groups were studied. Lipids and turbidity in serum at the fat tolerance test (before and 4 hours after the meal) were not associated with stature, weight or body surface area. Hence the use of a standard fat meal was justified.

The reproducibility of the lipid and turbidity measurements before and 4 hours after the fat meal was studied mainly in hypercholesterolaemias and hyperlipaemias and only in a small group of normocholesterolaemic normoglyceridaemic subjects (others). Fasting glyceride-glycerol varied by 22-23 per cent in hyper-

cholesterolaemias and in others while in hyperlipaemias it varied by 73 per cent. Corresponding values for turbidity were 80, 64 and 235 per cent. Glyceride-glycerol and turbidity in serum 4 hours after the fat meal were not associated with corresponding values in fasting serum that higher fasting values usually were accompanied by higher 4 hour values. If the relation between glyceride-glycerol in 4 hour serum and that in fasting serum was used as a measure of reproducibility of the fat tolerance test the error of the method was estimated to about 20 per cent.

Cholesterol in fasting serum at duplicate fat tolerance tests on unchanged diet varied by 11 per cent and phospholipids by 13 per cent. Cholesterol and phospholipid changes from fasting to 4 hour serum were greatest in that test where the fasting values were smallest. Serum cholesterol sometimes increased, sometimes decreased.

For the clinical groups however association was shown only between glyceride-glycerol in serum before and 4 hours after the fat meal but not for turbidity, cholesterol or phospholipids. The regression of glyceride-glycerol in serum 4 hours after the fat meal ( $y$ ) upon that of fasting serum ( $x$ ) was  $y = 1.6 \cdot x$  for the controls (male -

# REFERENCES

1. A. KATZ, E. H. & KUTCHER, M. D. The stabilization of serum lipid emulsions by serum phospholipids. *J. exp. Med.* 90: 409 1949.
2. ALBERT, M. J. Lipoprotein pattern as function of total triglyceride concentration of serum. *J. clin. Invest.* 40: 836, 1961.
3. ALBERT, M. J., MA, E. B. & PETERS, J. P. The relation of neutral fat to lactescence of serum. *J. clin. Invest.* 33: 147 1954.
4. ALBERT, M. & MA, E. B. Effect of carbohydrate ingestion on postprandial lipemia. *Clin. Res. Proc.* 4: 131 1966.
5. ALBERT, M. J. & YERGEN, R. S. Effect of previous starvation on the response of plasma lipids and free fatty acids to a meal. *J. clin. Invest.* 3: 461 1960.
6. ANDERVALL, G. Fettbelastung und Hydratierbarkeit. *Nord. Med.* 64: 1178 1960.
7. BAGE, I. Leber Lipemia 1-II. *Biochem. Z.* 91: 104 1918.
8. BARRETT, D. W. Alimentary lipemia in man with coronary artery disease and its control. *Brit. med. J.* 2: 640, 1956.
9. BECKER, G. H., MAYER, J. & KETTERER, H. F. Absorption and atherosclerosis. *Science.* 111: 329 1919.
10. BE, J. L., G. H. MAYER, J. & KETTERER, H. Fat absorption in young and old age. *Gastroenterology.* 14: 87 1930.
11. BERNOWITZ, D., LAROFF, W. & RUTTER, J. J. Serum triglyceride, esterified fat acid, and radiocarbon fat allowance studies. *Fed. Proc.* 18: 191 1959.
12. BILLIMORIA, J. D., DRYDALE, J. J. W., D. C. O. & MALARA, X. F. Determination of fibrinolytic activity of whole blood with special reference to the effect of exercise and fat feeding. *Lancet.* 110: 471 1959.
13. BIRN, H. J. & HERSCHBERG, H. L. Lactescence over lipemia II. *Hospitalstadiende.* 87: 29 1924.
14. BLIX, H. Studies on diabetic lipemia. Lund, AB PH. Lundstedts Univ. Bokhandel 1923.
15. BLOCK, W. J., BARKER, X. W. & MARY, F. D. Effect of small doses of heparin in increasing the transference of plasma during alimentary lipemia. *Circulation.* 4: 674 1951.
16. BLOOM, W. R. Fat emulsification. *J. biol. Chem.* 34: 447 1916.
17. BLOOMER, H. Blood fat tolerance tests in malnutrition and obesity. *Arch. Intern. Med.* 63: 121 1933.
18. BORN, K., GERTZ, V. B. & HOLMSTEDT, H. Investigation of the daily rhythm in the production of chylomicrons in man on the supply of fat. *Acta med. scand.* 106: 579 1941.
19. BOUTCHER, J. A. D. & BOUTCHER-STEWART, B. Alimentary lipemia and ischemic heart disease. *Lancet.* 7173: 343 1961.
20. BOUTCHER-STEWART, B. & BLACKBURN, R. (1956). The effect of corn oil on lipid clearance in patients with ischemic heart disease. In *Essential Fatty Acids*. ED. H. W. Scheele. Butterworths (London), p. 180.
21. BROWN, D. F., HENSLER, A. S. & DOTY, J. T. Postprandial lipemia in health and in ischemic heart disease. A comparison of three indexes of fat absorption and removal and their modification by systematic hepatic administration. *New Engl. J. Med.* 264: 732, 1961.
22. BRYN, G. Changes in the lipid contents of serum in patients with megal - dysproteinemia. *Acta psychiat. Scand.* 52: 1940.
23. CARLSON, L. A. Determination of serum glycerides. *Acta Soc. Med. Upsalen.* 64: 508, 1960.
24. CARLSON, L. A. Serum lipids in normal man. *Acta med. scand.* 167: 377 1960.

tween glyceride glycerol in the chylomicron phase of these groups so there must be a difference in chylomicron size (smaller in patients with atherosclerosis)

When lipids and turbidity of serum

were lowered by changing the diet from mainly saturated fats to mainly unsaturated fats a fat meal repeated on the latter diet showed fundamentally the same course but on a lower level

## ACKNOWLEDGMENTS

Throughout this investigation I have been much encouraged and stimulated by my colleague and friend Associate Professor BERTIL HOOD M D I thank him deeply I am also most grateful to the chief of Medical Department I Professor LARS WERKÖ M D for continuous generous support and for making available laboratory facilities My work has been greatly facilitated by my chief Assistant Professor TORSTEN LINDQVIST M D to whom I am much indebted Assistant Professor LENNART ÅNGERVALL, M D constructively criticized my work and merits my sincere thanks Mr ESDJÖRN CARLSTRÖM M A

rendered invaluable help with the statistical work. FOLKE HEYMAN M D assisted me with the interpretation of electrocardiograms.

I have pleasure in acknowledging my indebtedness to the ward nurses especially KERSTIN ÅHLQVIST INGA HWASS ETHEL HOOBERG Technical and clerical assistance was capably rendered by IVORID KARLSSON GUNNEL SVENSSON MARIANNE ÅHLSTRÖM and KAJSA ÅNGERVALL.

The investigation was supported by grants from the Swedish National Association Against Heart and Chest Diseases

- KRO, H. T. JOTNER, C. R. & REINHOLD J. D. Effects of fat ingestion and heparin administration on serum lipids of "Normal" hypercholesterolemia, hyperlipemia and atherosclerotic subjects. *Amer J med. Sci.* 222: 612, 1966.
- MALMCHOWA, R. Total serumcholesterol och cholesterol i elektroforetiskt skilda ämnen — och — betalipoproteinfractioner i ett norrmalmaterial. *Nord. Med.* 64 1187 1960
- MAN E. B. & ALBRINK, M. J. Serum lipids in different phases of carbohydrate metabolism. *Yale J Biol. Med.* 29: 216, 1936.
- MAN, E. B. & OILSTAD, E. F. The effect of ingestion of large amount of fat and of balanced meal on the blood lipids of normal man. *J. biol. Chem.* 90 61 1932.
- MARBER, L., BECKER, G. R., MARTEL, B. & SCHERER, H. Fat absorption and chylomicronemia. *Gastroenterology* 20: 43, 1952.
- MEHREZ, W. J. & POMEROY, Y. Influence of small doses of heparin on fat tolerance curves in vivo and in vitro. *Proc. Soc. exp. Biol.* 83: 164 1953.
- MITCHELL, J. R. A. & BROWNE-SWARTZ B. Abnormal lipemia and heparin clearing in megalic heart-disease. *Lancet* 1933: 167 1939
- MONROE J. R. Atherosclerosis and abnormal hyperlipemia. *Science* 109: 190, 1967
- MONROE J. R. Physical state of lipids and foreign substances producing atherosclerosis. *Science* 107 371 1946.
- MONROE J. R. Chylomicronemia, fat tolerance and atherosclerosis. *J. Lab. clin. Med.* 24: 373, 1930.
- NESTEL, P. J. DICKSON, M. A. & O'DRIS, J. Disposal of bismuth chylomicrons administered in intravenously in megalic heart disease and essential hyperlipemia. *Circulat. Res.* 10: 736, 1962.
- NEUMANN A. Ueber ultramikroskopische Blutuntersuchungen zur Zeit der Fettresorption bei Gesunden und Kranken. *Wien. klin. Woch.* 20: 681 1907
- NIELL, E. A. & KONTTINEN, A. Effect of physical activity on postprandial levels of fats in serum. *Lancet.* 1151 1962.
- NIEMI, N. L. Beitrag zur Betrachtung der alimentären Lipide des Menschen. *Acta med. scand.* 74 244, 1931
- OLSON, K. L., ZINN W. J. & WHARTON, O. K. Comparison of serum turbidity, chylomicronemia and total lipid values after fat test meal. *J. Amer. med. Ass.* 164: 633 1937
- POMEROY, J. BRIDFIELD W. H. & CHERRY M. Serum lipid and fat tolerance studies in normal, obese and atherosclerotic subjects. *Circulation.* 10: 742, 1954
- SCHWARTZ, L., WOLDOW A. & DUFRESNE, R. A. Determination of fat tolerance in patients with myocardial infarction. *J. Amer. med. Ass.* 144: 354 1933.
- SEIFER, H. & HADDE, W. Zur Frage der medikamentösen Beeinflussung der Serumlipide nach alimentärer Fettbelastung. *Z. ges. inn. Med.* 16 346, 1963
- SPENCER, H. W. Statistical Methods. Applied to experiments in agriculture and biology 4th ed. Ames. Iowa. 1934.
- SPERRY W. M. & SCHONHEIMER, R. A comparison of serum, heparinized plasma, and oxidized plasma in regard to cholesterol content. *J. biol. Chem.* 110: 633, 1935.
- SPERRY W. M. & WEBB, M. A review of the Schoenheimer — Sperry method for cholesterol determination. *J. biol. Chem.* 157 97 1930.
- SPERRY W. M. & WEBB, M. A. A review of the Schoenheimer-Sperry method for cholesterol determination. *J. biol. Chem.* 157 97 1930.
- SULLIVAN J. F. The effect of protein ingestion on elementary lipemia. *Amer J med. Sci.* 243- 770, 1962.
- S. ARNONE, A. & STEINBERG, L. Determination of unesterified fatty acids in normal human plasma. *Clin. chim. Acta.* 3: 443, 1966.
- S. ARNONE, A., STEINBERG, L. Plasma total lipid, cholesterol, triglycerides, phospholipids and free fatty acids in healthy Scandinavian population. *Acta med. scand.* 169: 43, 1961
- SWANE, R. L. & LEVY R. W. Chylomicron absorption dosage and size of action of heparin. *Am. J. Physiol.* 171 206, 1952.



- CARLSON L. A. & WADSTRÖM L. B.: On the occurrence of tri- and monoglycerides in human serum. Third Int. Conf on Biochemical Problems of Lipids. Brussels 1930 p. 123
- CARLSON L. A. & WADSTRÖM L. B.: Determination of glycerides in blood serum. 4 1 1939
- CONNOR H. & GOLDBERG C.: Effect of physical exercise on alimentary lipaemia. Brit. med J 2 509 1960.
- CRAMÉR L.: Serum  $\beta$  lipoprotein lipids and protein in normal subjects of different sex and age. Acta med scand 171:415 1960.
- CRAMÉR, K. & ISAKSSON B.: An evaluation of the Thorrell method for the determination of total serum cholesterol. Scand J clin Lab Invest. 11 13 1939
- DANFORTH, M. A.: Alimentary lipemia in ischemic heart disease. Clin. Sci 25:115 1963
- MCDONALD G. A. & FULLERTON H. W.: Effect of phenindion and bed rest on blood coagulability following a high fat intake. Lancet, ii 1111 1960
- EGGSTEIN M. & SCHREITLER, G.: The effect of feeding various fats on the levels of blood lipids. Proceedings of Fifth International Conference on the Biochemical Problems of Lipids. Essential Fatty Acids. 111 1938
- FRASER A. C. & STEWART H. C.: Ultra-microscopic particles in normal human blood. J Physiol (Lond) 90:18 1937
- GAGE, H. H. & FISH P. A.: Fat digestion, absorption, and excretion in man and animals as determined by the alkali field microscope and a fat-soluble dye. Amer J Anat 34 1 1924
- GROOVER, M. E. JR., JERRY AN J. I. and MARTIN C. D.: Variation in serum lipid concentration and clinical coronary disease. Amer J med Sc 55 133 1960
- GRUYER A. & HILDEBRAND T.: The occurrence of lipomilicrons in the blood of young and old individuals. Scand. J. in Lab Invest 31:236 1953
- HAVEL, R. J.: Early effects of fat ingestion on lipids and lipoproteins of serum in man. J clin Invest. 34 818 1953
- HEIKENTEN J., WAKO C. ANDLER ERO D. Fat loading studies in relation to age. Circulation 8 460 1953.
- HILLER, A. LINDER G. C. LUNDQVIST C. & VAN SLYKE D. D.: Fat metabolism in nephritis. J exp Med 30:331 1914
- HINSON H. F. & CARROLLARD L.: Serum esterified fatty acids with fat tolerance tests in Diabetes mellitus. Arch v Int Med 86 519 1930.
- HOOD B. & ANGERVALL, G.: The relation between inhibition towards clearing total cholesterol and distribution of cholesterol in plasma. Acta med scand 118.13 1937
- HOOD B. & ANGERVALL, G.: Studies in essential hypercholesterolemia and xanthomatosis. Relationships between sex, cholesterol concentration in plasma fractions and size of tendinous deposits. Circulation. 26 30 1939
- HORLICK, L.: The effect of a test meal of animal fat on the lipid and lipoproteins of human serum, and some aspect of liver function in normal individuals and in those with coronary atherosclerosis. Circulation 14 409 1956.
- HORLICK, L.: Effect of acute fat load on serum lipid in atherosclerosis. Circulat Res 4 368, 1957
- IDI S. M., POMPESIO P., COCCIMIAN & THEODORICO B.: La valeur de l'épreuve du repas gras dans l'héromélorose avec norme ou hypercholestérolémie. Sem. Hôp. 34 3908 1962
- JONES, R. J. & DORRILE: Factors influencing fat tolerance curve. Circulation 23 662, 1963
- KING BLAY H. J. & MORGAN D. M.: Basic types of lipid response. Clin Sc 12 845 1960
- KIN BURY K. J., MORGAN D. M. & BNEK VINGTOS P. C.: The effect of test load on the plasma lipids. Lancet 1915, 1950.
- KONTINEN A., RAJAN LMI M.: Effect of heavy cigarette smoking on postprandial triglycerides, free fatty acids, and cholesterol. Brit. med. J 1 830 1963
- KO P. T. & JOYNER, C. R.: Angina pectoris induced by fat ingestion in patient with coronary artery disease. J Amer med Ass. 14 1004 1933

1

- TALBOT G D & KEATING B M: Effects of preprandial whiskey on postalbuminary lipemia. *Geriatrics*. 17: 802, 1962.
- TANNHAUSER, S. J.: Serum lipids and their value in diagnosis. *New Engl J Med*. 237 516 1947
- THEORELL, H & WIDSTRÖM, G: Zur Methodik der Lipidanalysen im Blut unter besonderer Berücksichtigung des Gesamt-Cholesterins. *Zachr ges exper Med*. 75: 699 1931
- THEORELL, H.: Ueber quantitative Bestimmung der Lipide des aus Pfortenplasma ausgeschieden Eiweißkörper. *Biochem. Z*. 173: 296 1926
- VAN HANDEL, E., & SILVERMITH D B: Micro-method for the direct determination of serum triglycerides. *J Lab Med*. 50: 15., 1957
- WOLDOW A., CHAPMAN J E., EVAN J M: Fat tolerance in subjects with atherosclerosis. Heparin effects upon lipemia, lipoproteins, and gammaglobulina. *Amer Heart. J* 47 568, 1954.

# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 423

## AN ATROPHIC CIRCUMSCRIBED SKIN LESION IN THE LOWER EXTREMITIES OF DIABETICS

*by*

HANS MELIN

*Accompanied by 176*

---

UMEÅ 1964



# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 423

AN ATROPHIC CIRCUMSCRIBED SKIN  
LESION IN THE LOWER EXTREMITIES  
OF DIABETICS

by

HANS MELIN

*Accompanied by 176*

---

UMEÅ 1964

**GÖTEBORG 1964**  
**ÅLANDERS BOKTRYCKERI AKTIEBOLAG**

# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 423

AN ATROPHIC CIRCUMSCRIBED SKIN  
LESION IN THE LOWER EXTREMITIES  
OF DIABETICS

by

HANS MELIN

*Accompanied by 1 pl. 176*

---

UVEÅ 1964



**GÖTEBORG 1964**  
**KLANDERS BOKTRYCKERI AKTIEBOLAG**

FROM THE DEPARTMENT OF MEDICINE  
(HEAD PROFESSOR NILS TURNBLOM) UNIVERSITY OF UMEÅ, SWEDEN

---

# An atrophic circumscribed skin lesion in the lower extremities of diabetics

by

HANS MELIN

ALSO PUBLISHED AS SUPPLEMENT 423 TO  
ATCA MEDICA SCANDINAVICA

---

UMEÅ 1964

# ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv founded in 1869 by Axel Key. The first volume of Acta Medica Scandinavica is therefore numbered LII (52).

The chief editors have been Axel Key 1869—1900, C. G. Santesson 1901—1915, I. Holmgren 1916—1957 and Birger Strandell 1958 to date.

Acta Medica Scandinavica publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board. Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the Editor at the address below.

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that, if accepted, it will not be republished in any other journal in the same or similar form without the Editor's written consent.

Papers will be published in English, French or German at the authors' choice, followed by a summary in English.

The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left hand margin.

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author. No paper shall exceed 24 printed pages.

## Subscription

The annual subscription to the journal, covering two volumes, each of 6 numbers, is 140 Sw. crowns or U.S. \$ 27.25 including postage in the Scandinavian countries and in Holland 120 Sw. crowns.

*Address for subscriptions and all communications*  
ACTA MEDICA SCANDINAVICA  
P.O. Box 2052 Stockholm 2

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

FROM THE DEPARTMENT OF MEDICINE  
(HEAD PROFESSOR NILS TORNBLOM) UNIVERSITY OF UMEÅ, SWEDEN

---

An atrophic circumscribed skin lesion  
in the lower extremities of diabetics

by

HANS MELIN

ALSO PUBLISHED AS SUPPLEMENT 423 TO  
ATCA MEDICA SCANDINAVICA

---

UMEÅ 1964

TRANSLATED BY  
FREDERICK A. L. CHARLESWORTH

To my wife

TRANSLATED BY  
FREDERICK A. L. CHARLESWORTH

## PREFACE

The incentive to undertake this investigation came from my Chief Professor Nils Tönnblom, who first noticed the atrophic, circumscribed skin lesions and their association with diabetes and praediabetes.





## PREFACE

The incentive to undertake this investigation came from my Chief Professor Nils Törnblom, who first noticed the atrophic, circumscribed skin lesions and their association with diabetes and praedabetes.



## CONTENTS

I	Vascular disease in diabetes	9
II	Dermatologic lesions associated with diabetes	15
III	Atrophic circumscribed brownish skin lesions in the lower extremities of diabetics	17
IV	The parent material	21
V	Atrophic skin lesions in the lower extremities of patients without manifest diabetes	38
VI	Histopathological findings	41
VII	Microangiographic studies	45
VIII	The disappearance rate of radioactive iodide ( $I^{131}$ ) and sodium ( $Na^{24}$ ) in the atrophic skin lesions	48
IX	An investigation with the fluorescent antibody technique of the occurrence of gammaglobulin in the skin from diabetics by O. Larsson & H. Melin	56
X	Discussion	59
	Summary	62
	References	65



## CHAPTER I

## VASCULAR DISEASE IN DIABETES

The course and prognosis of diabetic disease to-day is practically dominated by vascular changes. Insulin brought about a radical change in the course of the disease and prolonged the span of life. Formerly diabetic coma was a common cause of death, whereas to day it is unusual that a diabetic succumbs to it. Instead it is the vascular changes and their kindred changes that prove decisive for the course of the disease, and resultant mortality thereof is much greater among diabetics in comparison with non-diabetics (Bell 1952). They also result in a more or less progressive invalidity that is accompanied by constant changes in the patient's social status.

Vascular changes in diabetes are partly located in the larger vessels, particularly in the coronary arteries and the arterial vessels of the legs, and partly in the small vessels e. g. the arterioles, the capillaries and the venules. The changes in the larger vessels are in congruence to those seen in arteriosclerosis and qualitatively cannot with certainty be separated from them. The changes in the small vessels are of an entirely different type and are often characteristic, if not specific, for diabetic disease. The small vessel disease in diabetes has, during the past two decades attracted more and more attention and it has even been emphasized as to whether it contributes to the arterioscle-

rosis in the larger vessels by affecting the vasa vasorum (cf. Warren and Le Compté 1952)

The changes in the small vessels have primarily been found in the eyes and kidneys. There is, however more and more proof for the supposition of the occurrence of a more or less generalized diabetic angiopathy (cf. Lundback 1949 1960). As a rule, diabetic disease is diagnosed several years prior to the vascular changes being clinically manifest. There is a pronounced positive correlation between the duration of diabetes and the frequency of retinopathy and nephropathy. A corresponding condition for the arteriosclerotic changes in the larger vessels is not so obvious. The latter appear to be more correlated to the patient's age (Lundback 1953 Liebow et al. 1955). The arteriosclerotic changes are considered to arise earlier and are more quantitatively pronounced than in corresponding age groups of non-diabetics (Lutz et al. 1942 Bell 1952). This has, however been repudiated in works by Goldenberg et al. (1958) and Blumenthal et al. (1960). They doubt if there is any difference in the intensity of arteriosclerosis in the aorta and the larger arteries in the extremities between diabetics and non-diabetics when the factors of age, sex and hypertension are properly assessed. Instead, they assert that there are

proliferative changes in the smaller vessels and find on an average that these changes are two and a half times more frequent in diabetics than in non-diabetics.

The histopathological changes in the small vessels appear best with PAS-staining (periodic acid Schiff) which suggests that they consist of mucoproteins and neutral mucopolysaccharides (McManus 1948). Through a direct chemical analysis of hyalinized glomeruli isolated from diabetic human kidneys with Kimmelsiel Wilson changes and Tornblom (1919) found no difference in this respect between diabetic and non-diabetic glomeruli. The amount was the same as in normal serum. In contradistinction to normal glomeruli the hyalinized glomeruli contained blood protein.

A brief survey will be given in the following of the most common diabetic complications considered to be connected with diabetic angiopathy.

#### DIABETIC RETINOPATHY

Diabetic retinopathy is often an early clinical indication of diabetic angiopathy. Diabetic retinopathy was first described by MacKenzie in 1877 and by Nettleship in 1888. They proved that the anatomic substratum of the small punctuated red dots seen at the ophthalmologic examination consisted of microaneurysms. The latter were rediscovered by Ballantyne in 1943. They could be demonstrated through the so-called flat preparation of the retina and by filling the retinal vessels with Indian ink, or by PAS-staining. They are located in the capillaries — chiefly to their venous

parts. The wall of the aneurysm often reveals a pronounced hyaline thickening, sometimes with endothelial proliferations and thrombotic formation (Ballantyne and Loewenstein 1944, Ballantyne 1945).

The genesis of the aneurysms is unknown. It is that of diabetic angiopathy in its entirety. The similarity with the capillary changes in the glomeruli in diabetic nephropathy has been pointed out (Ashton 1949). Diabetic retinopathy has certain similarities with a number of other eye diseases where tissue anoxia occurs with subsequent neovascularization (Wise 1956). Examples of such conditions are central vein thrombosis, periphlebitis retinae, malignant hypertension and sickle cell anaemia. Indications of microaneurysms have also been found in these conditions (Ballantyne and Loewenstein 1944, Wexler and Branover 1950). Microaneurysms have sometimes been found in entirely healthy eyes. They were however considerably less and situated in the periphery in contradistinction to diabetic retinopathy where they are larger and partial to the macular area (Ashton 1951). A dilatation of the retinal veins is also seen in the ophthalmoscopic picture. At later stages there is an increase in the number of aneurysms and the occurrence of deep hemorrhages and exudates. In the most advanced stadium the so-called proliferative stadium there is a new formation of vessels and connective tissue with preretinal hemorrhages and bleeding in the corpus vitreum.

Diabetic retinopathy may gradually lead to blindness and the prolonged duration of the disease has increased the frequency of blind diabetics. With a dia

betic duration of 20 to 30 years 10 to 15 per cent are blind (Lundback 1959 Winter 1960)

In rare cases a reddish discolouration is seen in the iris, *rubeosis iridis*. It is caused by a network of fine vessels on the frontal surface of the iris, the vessels probably being newly formed. Glaucoma often occurs in connection with rubeosis. In those cases where the eye-ground could be inspected a pronounced diabetic retinopathy is observed (Sales 1928 Kurz 1937). The incidence of rubeosis together with retinopathy indicates that rubeosis is conceived to be a diabetic vascular disease.

In a biomicroscopic study Ditzel (1954 1962) demonstrated changes in the bulbar conjunctival vessels in diabetes and above all a diminished relationship between the diameter of the arterioles and venules ( $A/V$  — ratio) of the larger pairs of vessels. He found that the changes appeared to be correlated to the duration of diabetes and that they also seemed to be more frequent in diabetes with retinopathy and nephropathy than in those without these complications. It was considered that the conjunctival vessel changes formed part of a specific diabetic angiopathy. The correlation to the diabetic duration and to the incidence of retinopathy and nephropathy has not, with certainty however been confirmed (Bech et al. 1960).

#### NELPHROPATHY

The expression diabetic nephropathy is often used to denote chronic kidney changes of vascular or mixed vascular and infectious genesis in diabetes. The

vascular changes are partly arterio-arteriosclerotic and partly capillary. The capillary changes were described by Kimmelstiel and Wilson (1936) who named them intercapillary glomerulosclerosis. It consists of a diffuse or a nodular form with hyalinization. The changes are stained with PAS (McManus 1948). The nodular form is generally considered specific for diabetes (Warren and Le Compte 1952 Bell 1953). Electronmicroscopic examinations have later supported the conception that the changes are primarily intracapillary (Bergstrand and Bucht 1957 Farquhar et al. 1959 Sabor et al. 1962).

Kimmelstiel and Wilson also reported a clinical syndrome characteristic of the so-called intercapillary glomerulosclerosis viz. the incidence of hypertension, massive proteinuria and oedema in diabetes. As a rule, the clinical picture is not so distinct, one or more of the symptoms may be lacking despite the characteristic patho-anatomical changes being pronounced. In general nephropathy is associated with a more or less pronounced albuminuria and slight hypertension and gradually leads to renal insufficiency with uremia. Nephropathy is always associated with retinopathy and similarly reveals a positive correlation to the diabetic duration (Goodof 1945 Ashton 1949 Wilson et al. 1951).

#### NEUROPATHY

In diabetes changes often occur in the peripheral nerves of the type of *polyneuritis*. In recent years more and more support has been given to the supposition that this is caused by changes in the



proliferative changes in the smaller vessels and find, on an average, that these changes are two and a half times more frequent in diabetics than in non-diabetics.

The histopathological changes in the small vessels appear best with PAS-staining (periodic acid Schiff) which suggests that they consist of mucoproteins and neutral mucopolysaccharides (McManus 1948) Through a direct chemical analysis of hyalinized glomeruli isolated from diabetic human kidneys with Kummelsiel-Wilson changes Odén and Törnblom (1959) found no difference in this respect between diabetic and non-diabetic glomeruli. The amount was the same as in normal serum. In contradistinction to normal glomeruli the hyalinized glomeruli contained blood protein.

A brief survey will be given in the following of the most common diabetic complications considered to be connected with diabetic angiopathy.

#### DIABETIC RETINOPATHY

Diabetic retinopathy is often an early clinical indication of diabetic angiopathy. Diabetic retinopathy was first described by MacKenzie in 1877 and by Nettleship in 1888. They proved that the anatomic substratum of the small punctuated red dots seen at the ophthalmologic examination consisted of microaneurysms. The latter were rediscovered by Ballantyne in 1943. They could be demonstrated through the so-called flat preparation of the retina and by filling the retinal vessels with Indian ink or by PAS-staining. They are located in the capillaries — chiefly to their venous

parts. The wall of the aneurysm often reveals a pronounced hyaline thickening sometimes with endothelial proliferations and thrombotic formation (Ballantyne and Loewenstein 1944, Ballantyne 1945).

The genesis of the aneurysms is unknown as is that of diabetic angiopathy in its entirety. The similarity with the capillary changes in the glomeruli in diabetic nephropathy has been pointed out (Ashton 1949). Diabetic retinopathy has certain similarities with a number of other eye diseases where tissue anoxia occurs with subsequent neovascularization (Wise 1956). Examples of such conditions are central vein thrombosis, periphlebitis retinae, malignant hypertension and sickle cell anaemia. Indications of microaneurysms have also been found in these conditions (Ballantyne and Loewenstein 1944, Wexler and Branover 1950). Microaneurysms have sometimes been found in entirely healthy eyes. They were, however, considerably less and situated in the periphery in contradistinction to diabetic retinopathy where they are larger and partial to the macular area (Ashton 1951). A dilatation of the retinal veins is also seen in the ophthalmoscopic picture. At later stages there is an increase in the number of aneurysms and the occurrence of deep hemorrhages and exudates. In the most advanced stadium the so-called proliferative stadium, there is a new formation of vessels and connective tissue with preretinal hemorrhages and bleeding in the corpus vitreum.

Diabetic retinopathy may gradually lead to blindness and the prolonged duration of the disease has increased the frequency of blind diabetics. With a dia

betic duration of 20 to 30 years 10 to 15 per cent are blind (Lundback 1959 Winter 1960).

In rare cases a reddish discolouration is seen in the iris, *rubeosis iridis*. It is caused by a network of fine vessels on the frontal surface of the iris, the vessels probably being newly formed. Glaucoma often occurs in connection with rubeosis. In those cases where the eye-ground could be inspected a pronounced diabetic retinopathy is observed (Salus 1928 Kutz 1937). The incidence of rubeosis together with retinopathy indicates that rubeosis is concerned to be a diabetic vascular disease.

In biomicroscopic study Ditzel (1954 1962) demonstrated changes in the bulbar conjunctival vessels in diabetics and above all a diminished relationship between the diameter of the arterioles and venules ( $A/V$  — ratio) of the larger pairs of vessels. He found that the changes appeared to be correlated to the duration of diabetes and that they also seemed to be more frequent in diabetics with retinopathy and nephropathy than in those without these complications. It was considered that the conjunctival vessel changes formed part of a specific diabetic angiopathy. The correlation to the diabetic duration and to the incidence of retinopathy and nephropathy has not, with certainty however been confirmed (Beck et al. 1963).

vascular changes are partly arterio-arteriosclerotic and partly capillary. The capillary changes were described by Kimmelstiel and Wilson (1936) who named them intercapillary glomerulosclerosis. It consists of a diffuse or a nodular form with hyalinization. The changes are stained with PAS (McManus 1948). The nodular form is generally considered specific for diabetes (Warren and Le Comptre 1952, Bell 1953). Electronmicroscopic examinations have later supported the conception that the changes are primarily intracapillary (Bergstrand and Bocht 1957 Farquhar et al. 1959 Sabor et al. 1962).

Kimmelstiel and Wilson also reported a clinical syndrome characteristic of the so-called intercapillary glomerulosclerosis viz. the incidence of hypertension, massive proteinuria and oedema in diabetics. As a rule the clinical picture is not so distinct, one or more of the symptoms may be lacking despite the characteristic patho-anatomical changes being pronounced. In general nephropathy is associated with a more or less pronounced albuminuria and slight hypertonia and gradually leads to renal insufficiency with uraemia. Nephropathy is always associated with retinopathy and similarly reveals a positive correlation to the diabetic duration (Goodof 1945 Ashton 1949 Wilson et al. 1951).

## NEPHROPATHY

The expression diabetic nephropathy is often used to denote chronic kidney changes of vascular or mixed vascular and infectious genesis in diabetics. The

## NEUROPATHY

In diabetes changes often occur in the peripheral nerves of the type of polyneuritis. In recent years more and more support has been given to the supposition that this is caused by changes in the

nutritive vessels vasa nervorum and consequently secondary to diabetic angiopathy (Woltman and Wilder 1929 Fagerberg 1959 Goldenberg et al. 1959)

As a rule a distinction is made between an acute reversible form that occurs in connection with severe ketoacidosis and a long term diabetic form that is not reversible. It is the latter which in the first place is usually denoted as diabetic neuropathy and which eventually is secondary to the changes in the vasa nervorum. It is often symmetric and located in the lower extremities. There may be a complete lack of symptoms or they may be distally notably pronounced. They often consist of paraesthesia or pains in the legs. The paraesthesia is experienced as tingling or burning and pains of a cramplike nature often occur at night. In severe cases lightning pains may occur. Neuropathy may be demonstrated in different degrees of severity. In the milder cases are found absent achilles reflexes diminished or normal patella reflexes or a slightly impaired sense of vibration. In the more severe cases are found absent achilles and patella reflexes a considerably reduced or absent sense of vibration and other disturbances in sensation and sometimes paresis.

Impaired autonomic function sometimes accompanies the neuropathy and the most common signs are nocturnal diarrhea orthostatic hypotension pupil lotonia deficient perspiration bladder atony and loss of potency (Goodman et al. 1953 Martin 1953 Aagaard 1962). A moderate elevated spinal fluid protein content without pleocytosis is often demonstrated. An increase of protein in

the spinal fluid may also be proved in diabetics without neuropathy but with vascular complications. The protein increase is presumed to be due to a change of the blood-brain barrier in all probability caused by diabetic vascular changes (Kuit et al. 1961).

The incidence of diabetic neuropathy varies considerably from 5 per cent (Rundles 1945) to 65 per cent (Root 1959) according to the composition of the material and the diagnostic criteria chosen. Diabetic neuropathy appears to be correlated to the diabetic duration and to the incidence of retinopathy and nephropathy (cf. Fagerberg 1959 Aagaard 1962 Steinness 1963).

Particularly in elderly badly controlled diabetics there sometimes occur bilateral asymmetric pareses with atrophy of the thigh musculature, especially of the quadriceps and pelvic girdle musculature diabetic amyotrophy. The condition develops during the course of some weeks and is associated with severe pain in the affected regions. The genesis is far from clear and is probably an expression of a metabolic disturbance of myogenic nature, or a combination of myogenic and neurogenic injury (Bischoff 1959 Locke et al. 1963). In muscle biopsies from patients with this disease endothelial proliferations in the small vessels have recently been demonstrated (Gärde and Kugelberg 1963).

#### CORONARY DISEASE

Coronary sclerosis and coronary thrombosis occur considerably more among diabetics than among non-diabetics. In a very exhaustive study of the incidence

of coronary artery disease from about 50,000 autopsies Clawson and Bell (1949) found that fatal coronary disease was about twice as frequent in diabetic as in non-diabetic males and three times as frequent in diabetic as in non-diabetic females. In agreement with other authors they also found that the incidence of disease of the coronary arteries in diabetic women was almost as high as in diabetic men, in contrast to the marked preponderance of the disease in non-diabetic men over non-diabetic women (Lundback 1953 Bradley and Bryfolge 1956)

The mortality after a coronary occlusion is also greater among diabetics than among non-diabetics (Clawson and Bell 1949). Coronary disease appears to be correlated to age as well as to diabetic duration (Bryfolge and Bradley 1957). Coronary vascular changes in diabetics might possibly be a combination of atherosclerosis in the larger vessels and for diabetes more or less specific changes in the small vessels (Warren and Le Compte 1952, Lundback 1960). Blumenthal et al. (1960) also found proliferative PAS-positive changes in the intramural coronary vessels. Diabetics revealed such lesions about two and a half times as frequently as non-diabetics. The highest frequency of proliferative lesions was found in the medium sized arteries. The lesions were histochemically similar to those seen in diabetic glomerulosclerosis and they did not find common arteriosclerotic lesions more frequently among diabetics than among non-diabetics.

## ARTERIAL DISEASE IN THE LOWER EXTREMITIES

It is evident that gangrene in the lower extremities is considerably more common in diabetics than in non-diabetics (Dry and Himes 1941 Bell 1950). Bell (1957) found that in patients over 40 years of age gangrene was about 50 times more common in diabetics than in non-diabetics, and that the ratio diabetic males to diabetic females was 1 to 1 in contrast to non-diabetics where it was 3 to 2. In Dry and Himes (1941) extensive series the same ratio for diabetic and non-diabetic men and women was 2 to 1 and 7 to 1 respectively. Diabetic gangrene has also other characteristics that differentiate it from the non-diabetic. It is often located in one or more toes, or is patchily distributed over the foot. The pulsations of the foot arteries are often preserved, and in many instances the prognosis may be good. The relation between the arterial disease in the lower extremities and diabetic duration is not as conspicuous as in those vascular changes previously mentioned e.g. the retinal and glomerular ones (Oakley et al. 1956 Bell 1957). There is a tendency to conceive the arterial disease in the lower extremities as a long-term diabetic symptom and as a combination of arteriosclerosis in the large and medium sized arteries and changes in the small vessels (Lundback 1960). Goldenberg et al. (1959) also support the idea of the incidence of a special diabetic angiopathy. Similarly to the findings in the coronary arteries they described endothelial proliferation and the deposit of a PAS-positive material in the small arteries and arterioles studied in amputated specimens.

nutritive vessels, vasa nervorum and consequently secondary to diabetic angiopathy (Woltman and Wilder 1929 Fagerberg 1959 Goldenberg et al 1959)

As a rule, a distinction is made between an acute reversible form that occurs in connection with severe ketoacidosis and a long term diabetic form that is not reversible. It is the latter which in the first place, is usually denoted as diabetic neuropathy and which eventually is secondary to the changes in the vasa nervorum. It is often symmetric and located in the lower extremities. There may be a complete lack of symptoms or they may be dissimilarly notably pronounced. They often consist of paraesthesia or pains in the legs. The paraesthesia is experienced as unling or burning and pains of a cramplike nature often occur at night. In severe cases lightning pains may occur. Neuropathy may be demonstrated in different degrees of severity. In the milder cases are found absent achilles reflexes diminished or normal patella reflexes or a slightly impaired sense of vibration. In the more severe cases are found absent achilles and patella reflexes, a considerably reduced or absent sense of vibration and other disturbances in sensation and sometimes paresis.

Impaired autonomic function sometimes accompanies the neuropathy and the most common signs are nocturnal diarrhoea, orthostatic hypotension, pupillomotoria, deficient perspiration, bladder atony and loss of potency (Goodman et al 1953 Martin 1953 Aagaard 1962). A moderate elevated spinal fluid protein content without pleocytosis is often demonstrated. An increase of protein in

the spinal fluid may also be proved in diabetics without neuropathy but with vascular complications. The protein increase is presumed to be due to a change of the blood-brain barrier in all probability caused by diabetic vascular changes (Kurt et al 1961).

The incidence of diabetic neuropathy varies considerably from 5 per cent (Rundles 1945) to 65 per cent (Root 1959) according to the composition of the material and the diagnostic criteria chosen. Diabetic neuropathy appears to be correlated to the diabetic duration and to the incidence of retinopathy and nephropathy (cf Fagerberg 1959 Aagaard 1962 Steiness 1963).

Particularly in elderly badly controlled diabetics there sometimes occur bilateral asymmetric pareses with atrophy of the thigh musculature especially of the quadriceps and pelvic girdle musculature, diabetic amyotrophy. The condition develops during the course of some weeks and is associated with severe pain in the affected regions. The genesis is far from clear and is probably an expression of a metabolic disturbance of myogenic nature, or a combination of myogenic and neurogenic injury (Bischhoff 1959 Locke et al 1963). In muscle biopsies from patients with this disease endothelial proliferations in the small vessels have recently been demonstrated (Gärde and Kugelberg 1963).

#### CORONARY DISEASE

Coronary sclerosis and coronary thrombosis occur considerably more among diabetics than among non-diabetics. In a very exhaustive study of the incidence

## CHAPTER II

## DERMATOLOGIC LESIONS ASSOCIATED WITH DIABETES

There is a heterogeneous group of skin lesions that may occur in connection with diabetes. Although none of them is absolutely pathognomonic of diabetes they arise more often in diabetics than in non-diabetics. Among those skin changes generally considered to be diabetic are necrobiosis lipodica diabetecorum and xanthoma diabetecorum.

Another group consists of bacterial and mycotic skin infections. They are considered to be more common among diabetics than among non-diabetics. Different causes of reduced resistance to infection have been discussed and inter alia the effect of the glucose concentration in the skin (Urbach 1945). The disposition to skin infections in connection with diabetes is considered to be due more to a poor general condition with malnutrition and dehydration as found in badly controlled diabetes (Marble 1959). A third group is generalized or localized pruritus which often appears in uncontrolled or poorly controlled diabetes. Finally changes may arise at the site of insulin injections, so-called lipodystrophy.

Necrobiosis lipodica diabetecorum consists of one or more distinctly demarcated, indurated bluish-red plaques with a smooth waxy surface generally located in the lower part of the leg. It usually begins as a papule that grows in size. In the later stages there is an extensive palm-

sized lesion with bluish-red, or brownish-red outer regions and a yellowish atrophic centre, at times with ulceration. As a rule it only gives cosmetic trouble. It occurs more often in young and middle aged women. This lesion of the skin was first observed by Oppenheim in 1929 but was named by Urbach in 1932. A similar change of the skin is also found among non-diabetics and Hare (1955) suggested the name necrobiosis lipodica without the additional diabetecorum for such cases. In a study covering a period of twenty years from the Mayo Clinic Rollins and Winkelmann (1960) found 111 cases with necrobiosis lipodica diabetecorum of which 29 were non-diabetics. Clinically the lesion in question appeared identical in diabetic and non-diabetic patients. Histologically however differences were found whereby it was suggested that the lesion in non-diabetics be named necrobiosis lipodica granulomatosa.

The histopathological findings in necrobiosis lipodica diabetecorum are areas of necrobiosis of the collagen throughout the dermis, especially in the lower parts. Within and near the areas of necrobiosis perivascular inflammatory infiltrates are found. The blood vessels in the dermis often exhibit fibrosis in the walls and proliferation in the endothelial lining occasionally with complete occlusion of

of the lower extremities in diabetes. The vascular lesions could be distinguished from arteriosclerosis lesions and were found in the vasa vasorum of the large vessels as well as in the small arteries of the nerves, muscles and skin. Similar findings are also reported by Lederman and Olson (1962). The symptom of intermittent claudication and objective signs of arterial insufficiency are also part of the picture of arterial disease in the lower extremities.

Similar diabetic vascular changes have also been reported concerning other vascular areas. Angervall et al (1961) thus found in a resection and biopsy material from diabetic stomachs small vessel changes characterized by a deposit of PAS-positive material in the walls and endothelial proliferation.

As an expression of a more generalized capillary injury an increased capillary fragility is found in diabetes. It is often combined with retinopathy (Hanum 1959, Rodriguez and Rona 1948). Circulation studies in diabetics with a short duration of the disease and without clinically proven diabetic vascular changes

have revealed abnormal vascular reaction. Although interpretations have varied it is presumed that changes have occurred in the small vessels and that there had already been an incidence of angiopathy at an early stage (Mogilnow et al 1953, Mendelsohn et al 1953, Párány 1955, Szegedi 1957, Lax and Léniksz 1957, Aagaard 1962).

Electron microscopic studies of particularly the capillary basement membrane in the renal glomeruli and in the retina as also in skin and muscle capillaries have revealed that the vascular changes may occur at an early stage in the course of the disease. Already in newly discovered diabetics of the maturity-onset type and in young diabetics and even in pre-diabetics changes of the capillary basement membrane have been found with manifold thickening (Daywig et al 1961, Aagaard and Hox 1961, Sabour et al 1962, Zach et al 1962).

The question has arisen as to whether they are secondary to the diabetic metabolic disorder or if they are primarily hereditary changes.

## CHAPTER II

## DERMATOLOGIC LESIONS ASSOCIATED WITH DIABETES

There is a heterogeneous group of skin lesions that may occur in connection with diabetes. Although none of them is absolutely pathognomonic of diabetes they arise more often in diabetes than in non-diabetics. Among those skin changes generally considered to be diabetic are *necrobiosis lipoidica diabetorum* and *xanthoma diabetorum*.

Another group consists of bacterial and mycotic skin infections. They are considered to be more common among diabetics than among non-diabetics. Different causes of reduced resistance to infection have been discussed and inter alia the effect of the glucose concentration in the skin (Urbach 1945). The disposition to skin infections in connection with diabetes is considered to be due more to poor general condition with malnutrition and dehydration as found in badly controlled diabetes (Marble 1959). A third group is generalized or localized pruritus which often appears in uncontrolled or poorly controlled diabetes. Finally changes may arise at the site of insulin injections, so-called lipodystrophy.

*Necrobiosis lipoidica diabetorum* consists of one or more distinctly demarcated, indurated bluish-red plaques with a smooth waxy surface generally located in the lower part of the leg. It usually begins as a papule that grows in size. In the later stages there is an extensive palm-

sized lesion with bluish-red, or brownish-red outer regions and a yellowish atrophic centre, at times with ulceration. As a rule it only gives cosmetic trouble. It occurs more often in young and middle aged women. This lesion of the skin was first observed by Oppenheim in 1929 but was named by Urbach in 1932. A similar change of the skin is also found among non-diabetics, and Hare (1955) suggested the name *necrobiosis lipoidica* without the additional *diabetorum* for such cases. In a study covering a period of twenty years from the Mayo Clinic Rollins and Winkelmann (1960) found 19 cases with *necrobiosis lipoidica diabetorum* of which 29 were non-diabetics. Clinically the lesion in question appeared identical in diabetic and non-diabetic patients. Histologically however differences were found whereby it was suggested that the lesion in non-diabetics be named *necrobiosis lipoidica granulomatosa*.

The histopathological findings in *necrobiosis lipoidica diabetorum* are areas of necrobiosis of the collagen throughout the dermis, especially in the lower parts. Within and near the areas of necrobiosis perivascular inflammatory infiltrates are found. The blood vessels in the dermis often exhibit fibrosis in the walls and proliferation in the endothelial lining, occasionally with complete occlusion of



of the lower extremities in diabetics. The vascular lesions could be distinguished from arterio-arteriosclerosis and were found in the vasa vasorum of the large vessels as well as in the small arteries of the nerves, muscles and skin. Similar findings are also reported by Pedersen and Olsen (1962). The symptom of intermittent claudication and objective signs of arterial insufficiency are also part of the picture of arterial disease in the lower extremities.

Similar diabetic vascular changes have also been reported concerning other vascular areas. Angervall *et al.* (1961) thus found in a resection and biopsy material from diabetic stomachs, small vessel changes characterized by a deposit of PAS-positive material in the walls and endothelial proliferations.

As an expression of a more generalized capillary injury an increased capillary fragility is found in diabetics. It is often combined with retinopathy (Hanum 1939, Rodriguez and Root 1948). Circulation studies in diabetics with a short duration of the disease and without clinically proven diabetic vascular changes

have revealed abnormal vascular reaction. Although interpretations have varied it is presumed that changes have occurred in the small vessels and that there had already been an incidence of angiopathy at an early stage (Megbow *et al.* 1953, Mendlowitz *et al.* 1953, Bárány 1955, Sigroth 1957, Lax and Feinberg 1959, Aagenaes 1962).

Electron microscopic studies of particularly the capillary basement membrane in the renal glomeruli and in the retina, as also in skin and muscle capillaries, have revealed that the vascular changes may occur at an early stage in the course of the disease. Already in newly discovered diabetics of the maturity onset type, and in young diabetics and even in pre-diabetic changes of the capillary basement membrane have been found with manifold thickening (Daysog *et al.* 1961, Aagenaes and Moe 1961, Sabour *et al.* 1962, Zacks *et al.* 1962).

The question has arisen as to whether they are secondary to the diabetic metabolic disorder or if they are primarily hereditary changes.

## CHAPTER III

## ATROPHIC CIRCUMSCRIBED BROWNISH SKIN LESIONS IN THE LOWER EXTREMITIES OF DIABETICS

Since 1960 a study of the incidence of a circumscribed atrophic brownish skin lesion in the lower extremities of diabetics has been carried out at the Medical Clinic in Umeå. Gradually we have become convinced that the lesion is more or less specific for diabetes. We consider that we have gained a rather good conception of the appearance and localization of the lesion. With the aid of colour photography it has been possible to follow the lesions during the course of time and thus gain a conception of their development and duration. As far as we know these lesions have not previously been described in the literature. By studying the illustrated material in papers on diabetes we feel that we have discovered them in pictures from patients illustrating other changes (Foley and Wright 1919, Lundback 1960).

The lesions were almost exclusively located in the lower parts of the legs. They consisted of small light brown atrophic lesions either isolated or grouped. Sometimes they were arranged in a linear pattern or more irregularly in groups. The lesions varied in size, but they seldom exceeded 10 mm in diameter. In shape they were most often round or rounded, at times more extended. The atrophy appeared as a depression in the skin surface corresponding to

the patch. The epidermis gave the impression of being thin and atrophic. The surface was often smooth and without crust. The atrophy varied from very distinct to hardly visible.

The lesions were brownish and clearly differed from the surrounding skin colour. The degree of pigmentation was correlated to the atrophy so that, as a rule, the most powerful pigmentation was found in the most pronounced atrophy. Where there was a less pronounced atrophy there was usually a lighter brown colour.

The lesions were localized in the front of the lower parts of the legs as well as on the medial and lateral sides. They were seldom found at the back of the legs. The lesions appeared on the bony parts of the legs as well as on the soft parts, and no definite distribution was found. The changes were often found on both legs, the one leg being sometimes more attacked than the other. The lesions were almost solely located in the lower parts of the legs and the feet were not affected. On single occasions the lesions have been found distal to the thigh. The lesions were not tender or itching. They are illustrated in Figs 6—14 at the end of the book.

As a result of repeated colour photography it has been possible to study the development of the lesions. It was found

the lumen Thrombosis in small vessels may even occur (Urbach 1932 Lever 1961)

The pathogenesis of necrobiosis lipoidica diabetorum is not known Hare (1955) did not consider it to be the result of an inflammation or a local lipoidosis The significance of the changes in the vessels has also been discussed and the possibility of secondary infiltration an imbibition of blood lipoids has been suggested (Urbach 1932 Prud Homme 1960) External trauma has also been mentioned as an exacerbating factor in several reports (Fitzpatrick 1960)

Xanthoma diabetorum is a rare lesion It is seen in patients with poorly controlled diabetes and is secondary to the hyperlipaemia The same lesions may also appear in other conditions with hyperlipemia The lesions often occur bilaterally as single or grouped papules and nodules of a yellowish colour on the buttocks, extensor surfaces palms and soles There may be marked pruritus with excoriations The lesions disappear with the treatment of diabetes mellitus including proper resutution of carbohydrate utilization (Pillsbury et al 1957 Fitzpatrick 1960)

It is often said that diabetics have a disposition for skin infections It is, however, considered that a properly treated diabetic has a normal resistance to skin infections (Fitzpatrick 1960)

The presence of mycotic infections of the vulva or in any area of the skin demands a search for diabetes mellitus and may occur in diabetics as eczematous

changes in intertriginous regions (Pillsbury et al. 1957) The incidence of dermatophytoses is probably not higher in diabetics than in non-diabetics However dermatophytosis of the interdigital spaces of the foot, causing epidermal fissures and erosions, may serve as a portal of entry for bacterial infections (Fitzpatrick 1960)

General pruritus may arise in diabetes There is, however, no connection between the itching and the severity of diabetes, and the former does not always vanish when the hyperglycemia and ketonuria are brought under control It has been suggested that the connection between the two conditions is merely coincidental (Guy 1951) Localized pruritus often occurs as pruritus vulvae in uncontrolled diabetes If it does not vanish when the diabetes is under control an examination may usually disclose some local cause such as infection or some other gynaecological disorder

Lipodystrophy is a change in the subcutaneous fat at the site of insulin injections It may develop a year or so after the commencement of insulin injections and may occur as a hypertrophy or an atrophy of the adipose tissue It is relatively frequent particularly in women and children The pathogenesis is not clear but it is presumed that the action of insulin at the site of injection is metabolic and facilitates the synthesis of fat from carbohydrate This should result in hypertrophy later followed by atrophy by a mechanism at present not known (Marble 1959)

11  
 10  
 9  
 8  
 7  
 6  
 5  
 4  
 3  
 2  
 1  
 0  
 1  
 2  
 3  
 4  
 5  
 6  
 7  
 8  
 9  
 10  
 11  
 12  
 13  
 14  
 15  
 16  
 17  
 18  
 19  
 20  
 21  
 22  
 23  
 24  
 25  
 26  
 27  
 28  
 29  
 30  
 31  
 32  
 33  
 34  
 35  
 36  
 37  
 38  
 39  
 40  
 41  
 42  
 43  
 44  
 45  
 46  
 47  
 48  
 49  
 50  
 51  
 52  
 53  
 54  
 55  
 56  
 57  
 58  
 59  
 60  
 61  
 62  
 63  
 64  
 65  
 66  
 67  
 68  
 69  
 70  
 71  
 72  
 73  
 74  
 75  
 76  
 77  
 78  
 79  
 80  
 81  
 82  
 83  
 84  
 85  
 86  
 87  
 88  
 89  
 90  
 91  
 92  
 93  
 94  
 95  
 96  
 97  
 98  
 99  
 100

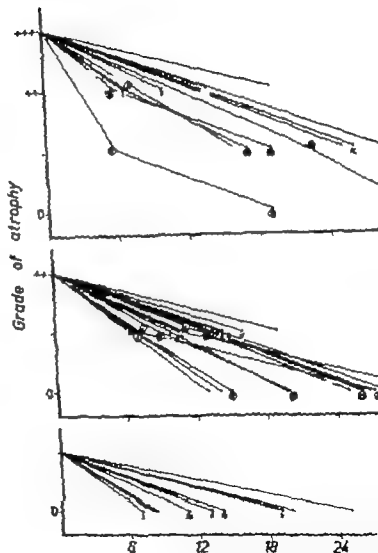


FIG 1 Duration of some selected atrophic skin lesions on the lower legs of patients studied by colour photography. Atrophy is graded in rough scale patients are numbered from 1 to 20. The circled figure denotes lesion at times.

1 B.M. 21 years fem. Diab 15 year

11 P.B. 64 years male.

2 S.L. 40 male Diab 20

12 R.M. 59 male

that the atrophy and pigmentation diminished almost parallelly and very slowly. On an average from one and a half to two years later the lesions had disappeared and only an insignificant atrophy distinguished them from their surroundings. The brownish colour had then also vanished and sometimes been replaced by a slight depigmentation. Sometimes the lesions had completely vanished. In the meanwhile new lesions might have arisen. The total picture therefore appeared unchanged as a rule. In reality the single skin lesion continuously changed towards a restitution. Fig. 1 schematically shows duration and restitution in some atrophic skin lesions observed in a series of technicolour pictures from 20 patients.

The patients were not often conscious of the existence of the lesions. Only a few patients made remarks concerning the type of occurrence. The majority of the patients whose attention was called to the changes presumed that they were traumatic without being able to determine any particular trauma. More often than not it went far back in time, which lessened the certainty of the statement. On the other hand some patients stated that they were subject to spontaneously occurring sores on their legs that took a long time to heal. Others again asserted that they easily got sores on their legs and considered them to have been caused by insignificant injuries.

Despite an intensive search it was difficult to determine the appearance of the primary lesion. Sometimes small superficial ulcers were found but they were probably several weeks old and statements regarding their occurrence were

as diffuse as they were varied. On one occasion we observed what we thought to be the primary lesion. It concerned a patient who on examination was seen to have atrophic skin lesions of the described type and who two days after the examination stated that he had spontaneously got a new rash on the legs. There were some intensively red macules of about the same size as the observed atrophic skin lesions and which did not disappear on pressure (Fig. 6a). Later they developed into typical atrophic lesions (Fig. 6b). In this case the type of incidence was spontaneous.

On some patients traumatization was performed with a hard rubber hammer but no changes arose. The question of the incidence type must yet remain unanswered.

The differential diagnosis of the atrophic skin lesions involve some other atrophic conditions of the skin. Papulonecrotic and acneiform tuberculids heal with the formation of circular sharply demarcated small scars, which are atrophic. They are often pale often with a slight pigmented border zone. They usually occur on the extremities and in the early stages may reveal histologically a tuberculoid structure (cf. Sutton 1956; Pillsbury et al 1957). The atrophic skin lesions in diabetics were only found on the lower extremities and papulonecrotic lesions were not observed. The atrophic lesions in diabetics disappear in contrast to the scars after healed tuberculids, and the histopathological picture of the lesions is not in agreement with that often seen in tuberculids (see page 43). Neurotic excoriations may appear as pigmented and sometimes slightly atrophic

## CHAPTER IV

### THE PATIENT MATERIAL

The material consisted of 293 patients with diabetes. A non-diabetic material used for comparison consisted of 104 patients with hypertension. All of them were admitted to the Medical Clinic in Umeå. All the patients with diagnosed diabetes who had received treatment during the period 1960—1962 were examined, and the comparative material was investigated consecutively during the same period (Melin 1962). A great number of the diabetic patients with previously described skin lesions were examined several times in order to study the course of the skin changes.

#### DIAGNOSTIC CRITERIA

All the diabetic patients were examined for so-called diabetic complications. With reference to retinopathy practically all the patients, and certainly all the doubtful cases, were examined by an ophthalmologist. The latter examined about 90 per cent of the cases, and the rest was examined by myself.

Retinopathy denotes the incidence of microaneurysms only or in association with haemorrhages, exudates and vascular proliferations. Patients with media opacities that prevented the ophthalmological examination were registered as cases without retinopathy. If the retinopathy was established previous to the

incidence of the media-opacities, the cases were however registered as retinopathy. Patients with advanced media-opacities, where the eye-ground could not be satisfactorily inspected, formed a relatively small part of the material — only 11 cases.

Nephropathy was registered if on repeated occasions, albuminuria arose without simultaneous signs of urinary tract infection. In those cases where there was a combination of albuminuria and urinary tract infection, the degree of albuminuria proved decisive for registration. Patients with a massive and constant albuminuria were registered as nephropathy. In those cases where there were also other albuminuric causal factors, such as cardiac decompensation or malignant hypertension, the diagnosis of diabetic nephropathy was, owing to natural reasons, uncertain. Doubtful cases have not been registered as nephropathy.

The frequency of neuropathy is partly dependent upon the criteria used for the diagnosis. The occurrence of subjective symptoms has not been dominant in the material. The dominant objective finding was a bilateral lack of achilles reflexes, and sometimes of the patella reflexes, too. A reduced, or lost vibration sense over the malleoli was also often observed. The vibration sense was tested with a tuning fork (C 64). Neuropathy was

lesions on any part of the body (Sutton 1956). They are often more irregular in shape than the lesions in diabetics and are an expression of a psychopathologic condition. In connection with chronic venous insufficiency in the lower extremities pigmented slight atrophic lesions are sometimes seen (Dodd and Cockert

1956). They are often isolated and are secondary to the varicose disease.

Other dermatologic diseases such as e. g. lichen planus often leave pigmented scars after healing. The case history and the distribution of the scars usually render the diagnosis of the primary disease clear.

Joslin et al 1959). The youngest patient was a 15 year old boy and the eldest a woman of 86 years of age. The median age for the men was 50.0 years, and for the women 60.2 years. The median age in the whole material was 55.3 years.

Of 130 men 78 had previously described atrophic skin lesions on the legs, whereas 10 had somewhat divergent skin changes. Of 163 women 46 had atrophic skin lesions on the lower legs, whereas 6 had somewhat divergent skin changes. In the continued studies of frequency the two groups with divergent skin changes were not included. The material consisted then of 120 men 78 of whom had atrophic skin lesions (65 per cent) and 157 women of whom 46 had atrophic skin lesions (29 per cent). The groups with divergent skin changes will later be dealt with separately.

The frequency of atrophic skin lesions in men and women was practically the same in the different age groups (Table 1). The youngest age group was an ex-

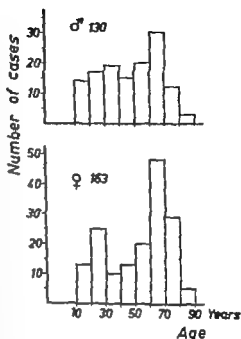


FIG 2 Age distribution of diabetic men and women.

TABLE 1 The frequency of atrophic skin lesions in different age groups in diabetic men and women

Men			Women		
Age	Cases	Per cent	Age	Cases	Per cent
10-19	2/13	15	10-19	1/13	8
20-29	12/16	75	20-29	8/23	35
30-39	12/18	67	30-39	5/9	56
40-49	10/14	71	40-49	3/12	25
50-59	11/18	61	50-59	5/18	28
60-69	20/28	71	60-69	18/48	38
70-79	10/11	91	70-79	5/29	17
80-89	1/2	50	80-89	1/5	20
Total	78/120	65	Total	46/157	29



registered where there was a bilateral lack of achilles reflexes with, or without a combination of weakened or extinct patella reflexes and reduced or lost sense of vibration. A prerequisite for only a double sided achilles reflex being registered as neuropathy was that no other reason for such areflexia existed and that the patient was without signs of ketonuria. Doubtful cases were not registered as neuropathy.

Hypertension was registered for diastolic pressure exceeding 100 mm Hg. The blood pressure was measured on several occasions and always during rest. In patients under 40 years of age a diastolic blood pressure exceeding 90 mm Hg was registered as hypertension.

Arterial disease in the lower extremities was assessed with the guidance of subjective symptoms in the form of intermittent claudication and objective findings such as the absence of palpable foot pulses and pallor of the foot when the leg was elevated. The incidence of gangrene or healed gangrene in the feet was similarly noted. That which was decisive for the diagnosis was subjective symptoms of intermittent claudication or the incidence of gangrene or the absence of palpable foot pulses in association with plantar blanching.

The atrophic skin lesions on the lower legs were registered with colour photography (Contarex, Zeiss-Ikon with Planar 1 2 f = 50 mm. and Sonar 1 2 f = 85 mm. Film Kodachrome Type A. Two Philips 500 watt lamps). The legs were partly photographed in toto and in part the skin changes were photographed at close range. The colour slides especially in doubtful cases, have been of

great help in the final analysis of the lesions.

The comparative material of patients with hypertension and without diabetes was examined in the same manner. The occurrence of atrophic skin changes on the legs corresponding to that observed in diabetics was registered. The incidence of hypertensive vascular changes in the retina according to Keith-Wagener was, in the majority of cases, assessed by an ophthalmologist. As malignant hypertension cases were registered with eye-ground changes of the 3rd and 4th degree and a diastolic blood pressure exceeding 130 mm Hg. Otherwise the examination was carried out in the same way as for the diabetic patients. The statistic significance was assessed according to the Chi Square method ( $= \chi^2$ ). Degrees of freedom (df) and significance limits for P are given. Yates's correction was used when samples were small (Guilford 1950).

#### THE DIABETIC MATERIAL

The diabetic material consisted of 130 men and 163 women. The distribution of the material in relation to sex and age groups is seen in Fig. 2. Among the women especially a double peak distribution was found with a maximum frequency in the group 20 to 29 years of age and one in the age group 60 to 69. The relation between men and women was about 1:1 and similarly in the different age groups with the exception of those age groups exceeding 60 years where the women dominated. A similar distribution has been demonstrated in other materials (Dahlberg et al 1947).

Joslin et al 1959). The youngest patient was a 15 year old boy and the eldest a woman of 86 years of age. The median age for the men was 50.0 years, and for the women 60.2 years. The median age in the whole material was 55.3 years.

Of 130 men 78 had previously described atrophic skin lesions on the legs, whereas 10 had somewhat divergent skin changes. Of 163 women 46 had atrophic skin lesions on the lower legs, whereas 6 had somewhat divergent skin changes. In the continued studies of frequency the two groups with divergent skin changes were not included. The material consisted then of 120 men 78 of whom had atrophic skin lesions (65 per cent), and 157 women of whom 46 had atrophic skin lesions (29 per cent). The groups with divergent skin changes will later be dealt with separately.

The frequency of atrophic skin lesions in men and women was practically the same in the different age groups (Table 1). The youngest age group was an ex-

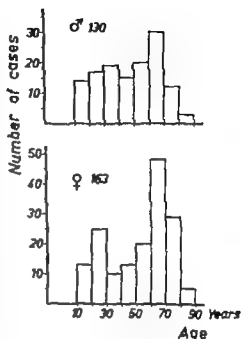


FIG. 2. Age distribution of diabetic men and women.

TABLE 1 The frequency of atrophic skin lesion in different age groups in diabetic men and women.

Men			Women		
Age	Cases	Per cent	Age	Cases	Per cent
10-19	2/13	15	10-19	1/13	8
20-29	12/16	75	20-29	8/25	32
30-39	12/18	67	30-39	5/9	56
40-49	10/14	71	40-49	3/12	25
50-59	11/18	61	50-59	5/18	28
60-69	20/28	71	60-69	18/48	38
70-79	10/11	91	70-79	5/29	17
80-89	1/2	50	80-89	1/5	20
Total	78/120	65	Total	46/157	29

registered where there was a bilateral lack of achilles reflexes with or without a combination of weakened, or extinct patella reflexes and reduced or lost sense of vibration. A prerequisite for only a double sided achilles reflex being registered as neuropathy was that no other reason for such areflexia existed and that the patient was without signs of ketonuria. Doubtful cases were not registered as neuropathy.

Hypertension was registered for diastolic pressure exceeding 100 mm. Hg. The blood pressure was measured on several occasions and always during rest. In patients under 40 years of age a diastolic blood pressure exceeding 90 mm Hg was registered as hypertension.

Arterial disease in the lower extremities was assessed with the guidance of subjective symptoms in the form of intermittent claudication and objective findings such as the absence of palpable foot pulses and pallor of the foot when the leg was elevated. The incidence of gangrene or healed gangrene in the feet was similarly noted. That which was decisive for the diagnosis was subjective symptoms of intermittent claudication or the incidence of gangrene or the absence of palpable foot pulses in association with plantar blanching.

The atrophic skin lesions on the lower legs were registered with colour photography (Contarex Zeiss-Ikon with Planar 1 2 f = 50 mm and Sonar 1 2 f = 85 mm Film Kodachrome Type A Two Philips 500 watt lamps). The legs were partly photographed in toto, and in part the skin changes were photographed at close range. The colour slides, especially in doubtful cases, have been of

great help in the final analysis of the lesions.

The comparative material of patients with hypertension and without diabetes was examined in the same manner. The occurrence of atrophic skin changes on the legs corresponding to that observed in diabetes was registered. The incidence of hypertensive vascular changes in the retina according to Keith-Wagener was in the majority of cases assessed by an ophthalmologist. As malignant hypertension cases were registered with eye-ground changes of the 3rd and 4th degree and a diastolic blood pressure exceeding 130 mm Hg. Otherwise the examination was carried out in the same way as for the diabetic patients. The statistic significance was assessed according to the Chi Square method ( $= \chi^2$ ). Degrees of freedom (df) and significance limits for P are given. Yates's correction was used when samples were small (Guilford 1950).

#### THE DIABETIC MATERIAL

The diabetic material consisted of 130 men and 163 women. The distribution of the material in relation to sex and age groups is seen in Fig. 2. Among the women especially a double peak distribution was found with a maximum frequency in the group 20 to 29 years of age and one in the age group 60 to 69. The relation between men and women was about 1:1 and similarly in the different age groups with the exception of those age groups exceeding 60 years where the women dominated. A similar distribution has been demonstrated in other materials (Dahlberg et al 1947).

than 10 years (Men  $\chi^2 = 23.4$  df = 1  $P < 0.001$  women  $\chi^2 = 15.0$  df = 1  $P < 0.001$ )

The frequency of atrophic skin lesions in relation to age and diabetic duration is seen in Table 3. It will be seen that the atrophic skin changes in the oldest age group occurred after a short diabetic duration in comparison with the youngest age group where they occurred only after a 10 year diabetic duration. Thus in the younger age groups there was a longer diabetic duration before the skin changes arose compared to the older groups. An increase of the atrophic skin lesions was, however, found in all the age groups with increasing diabetic duration.

In the oldest age group with a diabetic duration less than 5 years there were 22 patients with and 37 without atrophic skin lesions. Of the patients with atrophic skin changes 12 were men and 10 women. Of the 22 patients mentioned with atrophic skin changes 6 had retinopathy and in 3 there were media-opacities which made an examination of the retinal vessels impossible. None of the

37 patients without skin lesions had retinopathy. Among the latter however there were two patients with bilateral cataracts which prevented inspection. Judging from the frequency of retinopathy it would appear as if the patients with atrophic skin changes should have had a longer diabetes-duration than five years when compared with patients without these changes. It is well known that the onset of diabetes is difficult to determine in old age.

### Retinopathy

It is generally agreed that diabetic retinopathy is correlated to the duration of diabetes. As to whether diabetic retinopathy appears after a shorter duration of diabetes in elderly than in young diabetics is not quite clear. Dolger (1947) found that retinopathy occurs somewhat earlier in elderly diabetics in comparison to younger ones. He had, however, no patients over 50 years of age in his material. Pyke and Roberts (1959) found retinopathy in 64 per cent of elderly patients in connection with the discovery of their diabetes.

TABLE 3 The frequency of atrophic skin lesion in diabetic in relation to age and diabetic duration

Duration Years	< 10 yr		20-39 yr		Age groups 40-59 yr		> 60 yr		Total	
0-4	0/11	—	0/10	—	5/26	19	22/59	37	27/106	25
5-9	0/9	—	3/11	27	7/12	58	9/24	38	19/56	34
10-14	3/6	11	7/14	50	6/13	55	17/26	65	33/57	58
>15	—	—	27/31	90	11/13	85	7/14	50	45/58	78
Total	3/26	12	37/66	56	29/62	211	55/123	41	124/277	45

ception in that the frequency of skin changes was low for both men and women. In the oldest age groups the number of patients was small and thus the frequency figures were uncertain. There was no significant difference in frequency below and above the median age for men and women (Men  $\chi^2 = 0.916$   $df = 1$   $0.4 > P > 0.3$  Women  $\chi^2 = 0.279$   $df = 1$   $0.6 > P > 0.5$  Yates's correction).

If the frequency of skin lesions was calculated in relation to the duration of the diabetic disease instead it was seen that in men as well as in women the frequency of atrophic skin changes increased with an increasing diabetic duration (Table 2). The men had percentually a higher frequency of atrophic skin changes in the lower duration groups compared to the women. The frequency of atrophic skin changes was highest in the groups with a diabetic duration exceeding 15 years both for men and women; the difference in frequency between the sexes being less marked. The

number of patients in the longest duration groups was, however, small and therefore the frequency figures uncertain. Of the patients who had had diabetes for more than 30 years were 6 men, one of whom lacked skin lesions. He was a 66 year old man and had had diabetes for 31 years. With the exception of the last few years, when he was treated with sulphonylureas, he had been subject only to dietetic treatment. He showed no signs of diabetic complications. In the same duration group were 3 women, 2 of whom lacked skin changes. The one was 36 years old with a diabetic duration of 30 years and with only insignificant retinopathy. The other was 61 years old with a diabetic duration of 32 years and she, too, had only an insignificant retinopathy and a slight neuropathy.

The frequency of atrophic skin lesions after more than 10 years' diabetic duration was significantly higher than in patients with a diabetic duration of less

TABLE 2. The frequency of atrophic skin lesions in relation to diabetic duration in men and women.

Duration Years	Men Cases	Per cent	Duration Years	Women Cases	Per cent
0—4	17/43	40	0—4	10/63	16
5—9	10/18	56	5—9	9/38	24
10—14	22/27	82	10—14	11/30	37
15—19	16/18	89	15—19	7/11	64
20—24	5/5	100	20—24	6/10	60
25—29	3/3	100	25—29	2/2	100
> 30	3/6	83	> 30	1/3	33
Total	78/120	65	Total	46/157	29

than 10 years (Men  $\chi^2 = 23.4$  df = 1  
 $P < 0.001$  women  $\chi^2 = 15.0$  df = 1  
 $P < 0.001$ )

The frequency of atrophic skin lesions in relation to age and diabetic duration is seen in Table 3. It will be seen that the atrophic skin changes in the oldest age group occurred after a short diabetic duration in comparison with the youngest age group where they occurred only after a 10 year diabetic duration. Thus in the younger age groups there was a longer diabetic duration before the skin changes arose compared to the older groups. An increase of the atrophic skin lesions was, however, found in all the age groups with increasing diabetic duration.

In the oldest age group with a diabetic duration less than 5 years there were 22 patients with and 37 without atrophic skin lesions. Of the patients with atrophic skin changes 12 were men and 10 women. Of the 22 patients mentioned with atrophic skin changes 6 had retinopathy and in 3 there were media-opacities which made an examination of the retinal vessels impossible. None of the

37 patients without skin lesions had retinopathy. Among the latter however there were two patients with bilateral cataracts which prevented inspection. Judging from the frequency of retinopathy it would appear as if the patients with atrophic skin changes should have had a longer diabetes-duration than five years when compared with patients without these changes. It is well known that the onset of diabetes is difficult to determine in old age.

### Retinopathy

It is generally agreed that diabetic retinopathy is correlated to the duration of diabetes. As to whether diabetic retinopathy appears after a shorter duration of diabetes in elderly than in young diabetics is not quite clear. Dolger (1947) found that retinopathy occurs somewhat earlier in elderly diabetics in comparison to younger ones. He had, however, no patients over 50 years of age in his material. Pyke and Roberts (1959) found retinopathy in 6.4 per cent of elderly patients in connection with the discovery of their diabetes.

TABLE 3 The frequency of atrophic skin lesion in diabetic in relation to age and diabetic duration

Duration Years	< 20		20—39		Age groups 40—59		> 60		Total	
	n	%	n	%	n	%	n	%	n	%
0—4	0/11	—	0/10	—	1/26	19	22/19	57	27/106	25
5—9	0/9	—	3/11	27	7/12	58	9/24	38	19/76	25
10—14	3/6	50	7/14	50	6/11	55	17/26	65	33/57	58
> 15	—	—	27/53	51	11/13	85	7/14	50	45/58	78
Total	3/26	12	37/66	56	29/62	47	55/123	45	126/277	45

The frequency of retinopathy in relation to age and diabetic duration in this material of diabetic patients is seen in Table 4. It will be seen also here that the diabetic duration previous to the onset of retinopathy decreased with increasing age. In the youngest age group retinopathy only arose after a 10 year diabetic duration whereas in the oldest age group it was found in 10 per cent in the group with less than 5 years diabetic duration. In the total column to the right of the Table it will be seen also how the frequency of retinopathy increased with increasing diabetic duration. After more than 15 years of diabetic duration so-called long term diabetes, the frequency of retinopathy was 90

per cent. This figure is rather high in comparison with other materials (Dolger 1947 Mårtensson 1950 Lundback 1953). Lundback found in a long-term material from Aarhus in Denmark a frequency of retinopathy of 80 per cent, while Mårtensson in a similar Swedish material only found a frequency of 40 per cent. The highest frequency 100 per cent is reported from the U.S.A. by Dolger on diabetics with a duration of diabetes of up to twenty five years.

The frequency of retinopathy in men and women with and without atrophic skin lesions respectively is seen in Table 5. There was a total of 86 patients (69 per cent) with retinopathy in the group with atrophic skin changes as compared

TABLE 4 The frequency of retinopathy in diabetics in relation to age and diabetic duration.

Duration Years	Age groups								Total %	
	< 20 %		20—39 %		40—59 %		> 60 %			
0—4	0/11	—	0/10	—	2/26	8	6/19	10	8/106	8
5—9	0/9	—	5/11	46	7/12	58	9/24	38	21/56	38
10—14	5/6	83	12/14	86	8/11	73	18/26	69	43/57	75
>15	—	—	31/31	100	11/13	85	10/14	71	52/58	90
Total	5/26	19	48/66	73	28/62	45	43/123	35	124/277	45

TABLE 5 The frequency of retinopathy in diabetic men and women with and without atrophic skin lesions

	With Cases %		Without Cases %		Total Cases %	
Men	54/78	69	6/42	14	60/120	50
Women	32/46	70	32/111	29	64/157	41
Total	86/124	69	38/153	25	124/277	45

with 38 patients (25 per cent) in the group without such lesions. The difference in frequency of retinopathy between the groups with and without atrophic skin changes was significant for both men and women (Men  $\chi^2 = 33.0$  df = 1  $P < 0.001$  Women  $\chi^2 = 22.4$  df = 1  $P < 0.001$ ).

Exudative and proliferative retinopathy was found in 39 per cent (48 patients) in the group with atrophic skin lesions as against only 9.8 per cent (15 patients) in the group without such lesions. The ratio of the total frequency of retinopathy in the group with and without skin changes was 69:25 or 2.8:1. The corresponding ratio for the advanced retinopathy was 39:9.8 or 4:1. Proportionately there were thus more patients with advanced retinopathy in the group with atrophic skin changes than in the one without. The difference was probably significant ( $\chi^2 = 3.30$  df = 1  $0.1 > P > 0.05$  Yates's correction).

#### *Diabetic nephropathy*

Diabetic nephropathy is, like retinopathy, correlated to the diabetic duration and is considered to occur with

about the same frequency in men and women. It has sometimes been reported that there is a predominance among men in the younger age groups (1953).

There were 41 patients (15 per cent) with nephropathy in this material. The frequency was about the same for men and women, 17 and 13 per cent respectively (Table 6). The frequency of nephropathy in the group with atrophic lesions was significantly greater than in the group without ( $\chi^2 = 24.8$  df = 1  $P < 0.001$ ).

The frequency of nephropathy in relation to age and diabetic duration is in Table 7. The same tendency was observed here as in diabetic retinopathy. Previous to the onset of nephropathy, diabetic duration decreased with increasing age. In the oldest age group was a patient with nephropathy in the lowest duration group, while in the youngest age group there were 2 patients with nephropathy only at a 10 year diabetic duration. The number of cases in the different duration groups was small and permitted a crude assessment of the frequency

TABLE 6 *The frequency of nephropathy in diabetic men and women with and without atrophic lesions.*



The frequency of retinopathy in relation to age and diabetic duration in this material of diabetic patients is seen in Table 4. It will be seen also here that the diabetic duration previous to the onset of retinopathy decreased with increasing age. In the youngest age group retinopathy only arose after a 10 year diabetic duration whereas in the oldest age group it was found in 10 per cent in the group with less than 5 years diabetic duration. In the total column to the right of the Table it will be seen also how the frequency of retinopathy increased with increasing diabetic duration. After more than 15 years of diabetic duration so-called long term diabetes, the frequency of retinopathy was 90

per cent. This figure is rather high in comparison with other materials (Dolger 1947 Mårtensson 1950 Lundback 1953). Lundback found in a long-term material from Aarhus in Denmark a frequency of retinopathy of 80 per cent while Mårtensson in a similar Swedish material only found a frequency of 40 per cent. The highest frequency, 100 per cent is reported from the U S A by Dolger on diabetics with a duration of diabetes of up to twenty-five years.

The frequency of retinopathy in men and women with and without atrophic skin lesions respectively is seen in Table 5. There was a total of 86 patients (69 per cent) with retinopathy in the group with atrophic skin changes as compared

TABLE 4 The frequency of retinopathy in diabetic in relation to age and diabetic duration

Duration Years	Age groups								Total n	
	< 20 %		20—39 %		40—59 %		> 60 %			
0—4	0/11	—	0/10	—	2/26	8	6/59	10	8/106	8
5—9	0/9	—	5/11	46	7/12	58	9/24	38	21/56	38
10—14	5/6	83	12/14	86	8/11	73	18/26	69	43/57	75
> 15	—	—	31/31	100	11/13	85	10/14	71	52/58	90
Total	5/26	19	48/66	73	28/62	45	43/123	35	124/277	45

TABLE 5 The frequency of retinopathy in diabetic men and women with and without atrophic skin lesions

	With Cases %		Without Cases %		Total Cases %	
Men	54/78	69	6/42	14	60/120	50
Women	32/46	70	32/133	29	64/179	41
Total	86/124	69	38/175	25	124/277	45

TABLE 1 The frequency of neuropathy in diabetic men and women with and without atrophic skin lesions.

	With		Without		Total	
	Cases	n	Cases	n	Cases	n
Men	47/78	60	5/42	12	52/120	43
Women	23/46	50	21/111	23	44/157	31
Total	70/124	17	30/153	20	100/277	36

men  $\chi^2$  10.3 df 1  $0.005 > P > 0.001$  Yates's correction). The frequency of neuropathy for the whole material was 36 per cent, for the men 43 per cent, for the women 31 per cent. The men had a somewhat higher frequency of neuropathy than the women. ( $\chi^2$  = 4.80. df 1  $0.05 > P > 0.03$ ) As previously mentioned the frequency figures vary for different materials, but largely they range from about 40 to 65 per cent (cf Steiness 1963). In two investigations of diabetic neuropathy carried out by Fagerberg (1959) and Steiness (1963) the corresponding figures were 63 and 52 per cent respectively. In a long-term material with more than 15 years' duration Lundback (1953) found only 32 per cent with achilles areflexia. If weak achilles reflexes also were included the figure rose to 45 per cent.

The frequency of neuropathy in relation to age and diabetic duration is given in Table 9. As is seen in the lower row in the Table the total frequency of neuropathy was low in the youngest age group in comparison with the remainder. The number of patients in this group was, however, small, and there were comparatively few cases with a diabetic duration exceeding 10 years. In the other

age groups the frequency of neuropathy was about as great. It has been stated that the frequency of diabetic neuropathy is greater in patients over 60 years of age than in those below this age limit (Jordan 1936 Fagerberg 1959 Steiness 1963). No difference in this respect is found in this material ( $\chi^2$  = 0.023 df = 1  $0.9 > P > 0.8$ ).

In all the age groups the frequency of neuropathy increased with increasing diabetic duration. The total frequency of neuropathy in the different duration groups is given in the right column of the Table. The frequency rose evenly with increasing diabetic duration except in the group with the longest duration. The frequency in this group increased only inconsiderably compared to the duration group 10 to 14 years. Steiness (1963) found in Lundback's material a similar conclusion in the groups with 15 to 19 and 20 to 25 years' duration. Discussions have taken place as to whether the frequency of diabetic neuropathy becomes constant after a certain diabetic duration, or if some selective factor is in action (Steiness 1963).

Earlier in the presentation a division was made of the frequency of retino- and nephropathy in relation to age and

TABLE 7 The frequency of nephropathy in diabetics in relation to age and diabetic duration

Duration Years	< 20		20-39		Age groups 40-59		> 60		Total	
	n/		n/		n/		n/		n/	
0-4	0/11	—	0/10	—	0/26	—	1/59	2	1/106	1
5-9	0/9	—	0/11	—	1/1	8	1/24	4	2/56	4
10-14	2/6	33	5/14	36	1/11	9	6/26	23	14/57	25
≥ 15	—	—	19/31	61	3/13	23	2/14	14	24/58	40
Total	2/26	8	24/66	39	5/62	8	10/123	8	41/277	15

total frequency in the different duration groups is seen in the right column of the Table. It increased evenly with increasing diabetic duration. Of the 58 patients with a diabetic duration exceeding 15 years — long-term diabetes — 24 (41 per cent) had nephropathy. In long-term diabetic materials studied by Mårtensson (1950) and Lundbaek (1953) the corresponding figures were 33 and 58 per cent respectively.

The frequency of nephropathy in this material in the different age groups appeared to be higher in the two lowest and if the material was divided into two groups, under and over 40 years, the frequency of nephropathy was significantly higher in the younger than in the older group ( $\chi^2 = 18.3$  df = 1  $P < 0.001$  Yates's correction). This may be partly due to the fact that nephropathy in the older age groups may often be blurred by urinary tract infection hence the clinical picture being more difficult to assess. Moreover, per centually there were more patients with long-term diabetic duration in the younger group than in the older one.

The fact that nephropathy gives rise to a poor prognosis was seen in that 33

of the 41 cases with nephropathy died during the period of examination. Post mortem examinations were performed on 9 of these patients and in 7 of them glomerulosclerotic changes were established according to Kimmelstiel-Wilson. The remainder had nephrosclerosis without signs of glomerulosclerosis. With the exception of one all the patients had previous to death signs of increasing renal insufficiency with uraemia.

### Neuropathy

More and more support has been given to the opinion that diabetic neuropathy has a vascular origin. It has been proved that it is correlated to diabetic duration and to other so-called diabetic complications, above all retinopathy and nephropathy (cf. Fagerberg 1959).

The frequency of neuropathy in patients with and without atrophic skin lesions is seen in Table 8. There is a considerably greater frequency of neuropathy in patients with atrophic skin changes, men as well as women compared with patients without such changes. The difference is significant for both men and women. (Men  $\chi^2 = 24.1$  df = 1  $P < 0.001$  Yates's correction. Wo-

TABLE 1 The frequency of neuropathy in diabetic men and women with and without atrophic skin lesions.

	With Cases		Without Cases		Total Cases	
		n		n		n
Men	47/78	60	5/42	12	52/120	43
Women	25/46	50	25/111	23	48/157	31
Total	72/124	57	30/153	25	102/277	36

men  $\chi^2 = 10.3$  df = 1  $0.005 > P > 0.001$  Yates's correction) The frequency of neuropathy for the whole material was 36 per cent, for the men 43 per cent, for the women 31 per cent. The men had a somewhat higher frequency of neuropathy than the women ( $\chi^2 = 4.80$  df = 1  $0.05 > P > 0.03$ ). As previously mentioned the frequency figures vary for different materials, but largely they range from about 40 to 65 per cent (cf Stenness 1963). In two investigations of diabetic neuropathy carried out by Fagerberg (1959) and Stenness (1963) the corresponding figures were 63 and 52 per cent respectively. In a long-term material with more than 15 years' duration Lundback (1953) found only 32 per cent with achilles areflexia. If weak achilles reflexes also were included the figure rose to 43 per cent.

The frequency of neuropathy in relation to age and diabetic duration is given in Table 9. As is seen in the lower row in the Table the total frequency of neuropathy was low in the youngest age group in comparison with the remainder. The number of patients in this group was, however, small, and there were comparatively few cases with a diabetic duration exceeding 10 years. In the other

age groups the frequency of neuropathy was about as great. It has been stated that the frequency of diabetic neuropathy is greater in patients over 60 years of age than in those below this age limit (Jordan 1936, Fagerberg 1959, Stenness 1963). No difference in this respect is found in this material. ( $\chi^2 = 0.023$  df = 1  $0.9 > P > 0.8$ )

In all the age groups the frequency of neuropathy increased with increasing diabetic duration. The total frequency of neuropathy in the different duration groups is given in the right column of the Table. The frequency rose evenly with increasing diabetic duration except in the group with the longest duration. The frequency in this group increased only inconsiderably compared to the duration group 10 to 14 years. Stenness (1963) found in Lundback's material a similar condition in the groups with 15 to 19 and 20 to 25 years' duration. Discussions have taken place as to whether the frequency of diabetic neuropathy becomes constant after a certain diabetic duration, or if some selective factor is in action (Stenness 1963).

Earlier in the presentation a division was made of the frequency of retinopathy and nephropathy in relation to age and

TABLE 9 *The frequency of neuropathy in diabetics in relation to age and diabetic duration*

Duration Years	< 20		20-39		Age groups 40-59		> 60		Total	
	%		%		%		%		%	
0-4	0/11	—	0/10	—	3/26	12	15/59	25	18/106	17
5-9	2/9	22	1/11	9	5/12	42	8/24	33	16/56	29
10-14	3/6	50	9/14	64	5/11	46	15/26	58	32/57	56
>15	—	—	17/31	55	10/13	77	7/14	50	34/58	59
Total	5/26	19	27/66	41	23/62	37	45/123	37	100/277	36

diabetic duration whereby it was seen that diabetic duration previous to the onset of those complications was longest in the youngest age group but subsequently abated with increasing age and that in the oldest age group there was already a number of cases with complications in connection with the onset of diabetes. A corresponding condition was found for neuropathy but not so pronounced. In the youngest age group neuropathy arose already after a 5 year diabetic duration and in the oldest age group there were 15 patients with neuropathy prior to a 5 year diabetic duration. The number of cases was however small and did not permit a closer interpretation. As a possible alternative to diabetic neuropathy occurring earlier than other so-called complications it is possible that the diagnostic criteria have not been adequate or that apart from the vascular origin there may be a contributory nonduration-conditioned factor. Neuropathy can lead to the discovery of diabetic disease in higher ages, or it may occur early after the onset of diabetes. (Rundles 1945; Martin 1953) In the oldest group with less than 5 years dia-

betic duration neuropathy was found in one fourth of the patients.

In the majority of cases diabetic neuropathy gave little or no subjective symptoms and where such existed it was often difficult to decide if it was due or not to neuropathy. In 13 (10 men and 3 women) of the 100 cases with neuropathy this was more pronounced with severe subjective symptoms often with uncertainty in the gait and fear of faulty stepping. The objective findings in this group were also more pronounced with lost achilles and patella reflexes, loss of vibration, impaired sensibility and sometimes paresthesia. These cases were classified severe. They occurred in all age groups with the exception of the youngest and appeared to be somewhat more common below than above 60 years of age (9 and 4 cases respectively). Seven of the 13 cases with severe neuropathy had a diabetic duration exceeding 15 years, the remainder being evenly distributed in the lower duration groups. With the exception of one all the patients had atrophic skin lesions on the legs. One might suppose that in this group neuropathy was of significance as regards the

occurrence of the skin changes, particularly by way of a traumatic origin. The group was, however small and the severe neuropathy could therefore hardly be of any decisive importance to the incidence of the atrophic skin lesions on the whole.

#### *Arterial disease in the lower extremities*

In the material there were 38 patients (14 per cent) with arterial disease in the lower extremities either in the form of gangrene in the feet, or in the form of intermittent claudication with obvious signs of arterial insufficiency.

Table 10 shows the frequency of arterial disease in the lower extremities in men and women with and without atrop-

hic skin lesions. There was a total of 20 per cent in the group with atrophic skin changes compared with 9 per cent in the group without such changes. The difference was significant ( $\chi^2$  6.92, df = 1,  $0.01 > P > 0.005$  Yates correction). In the group without atrophic skin changes the frequency difference of arterial disease was great between men and women — 11 and 12 per cent respectively. The difference was due to the men in this group being considerably younger than the women. The frequency of arterial disease of the lower extremities in the whole material was 14 per cent and here there was no difference between men and women. This similarity in the frequency of peripheral arterial disease

TABLE 10 *The frequency of arterial disease in the lower extremities in diabetic men and women with and without atrophic skin lesions*

	With		Without		Total	
	Cases	n	Cases	n	Cases	n
Men	17/73	23	0/42	—	17/120	14
Women	8/46	17	13/111	12	21/157	13
Total	25/124	29	13/153	9	38/277	14

TABLE 11 *The frequency of peripheral arterial disease in the lower extremities in diabetic in relation to age and diabetic duration*

Duration Years	<60		Age groups >60		Total	
		n		n		n
0—4	4/47	9	10/59	17	14/106	13
5—9	3/52	9	2/24	8	5/76	9
10—14	2/31	6	10/26	38	12/57	21
>15	5/44	11	2/14	14	7/58	12
Total	14/154	9	24/123	20	38/277	14

in diabetic men and women has often been put forward and differs considerably from corresponding conditions in non-diabetic men and women where the former are extremely dominant (Dry and Hines 1941 Bell 1957)

The frequency of arterial disease in the lower extremities in relation to age and diabetic duration is given in Table 11. The smallness of the material only permitted a division into two age groups below and above 60 years of age. As was to be expected there was a considerably higher frequency of peripheral arterial disease in the older age group in comparison with the younger one. Only one patient was under 40 years of age viz a 36 year old man with a diabetic duration of 29 years and gangrene in the foot. The distribution between men and women in the two duration age groups was about the same. Percentually there was some preponderance for the women in the younger group, and for the men in the older one. There was no definite increase of the total frequency of arterial disease in the lower extremities with increasing diabetic duration. Here an unavoidable selection of the material is of consequence in that the patients with diabetic gangrene often come under the care of a surgeon. However it was possible to ascertain that arterial disease of the lower extremities even in the age group below 60 could occur early after the onset of diabetes. With the exception of one all the patients were over 40 years of age and, as previously pointed out it is probable that there are several pathogenetic factors in arterial disease such as e.g. arteriosclerosis in the large vessels in combination with the

previously described changes in the small vessels.

Twenty-eight patients had gangrene located in one or more toes or in the heel and the sole of the foot. Of these 20 (71 per cent) had atrophic skin lesions on the leg. Nine patients, or about one third had palpable pulses on the gangrenous foot. This is in agreement with the general conception that patients with diabetic gangrene often have retained foot pulses.

Fourteen patients with gangrene were examined with femoral angiography. Six patients had normal angiograms where the vessels could be followed right down to the foot. In all these cases the foot pulses were also palpable.

These 6 patients with diabetic gangrene were of special interest in that they had a normal angiogram and normal foot pulses. Their ages ranged from 45 to 69. Three of them had atrophic skin lesions on the legs. Four of them had neuropathy to a slight degree with achilles and patellar areflexia and with a slight reduced sense of vibration but without reduced superficial sensibility. The normal angiographic finding and the absence of severe neuropathy support the conception that a part of the origin of diabetic gangrene consists of changes in the small vessels. An impaired circulation through the large arteries may further facilitate the incidence of gangrene and prove prognostically unfavourable. As previously seen the frequency of arterial disease in the lower extremities was significantly correlated to the incidence of atrophic skin lesions. But as arterial disease in the lower extremities only occurred in 14 per cent of the whole material

it could not be of any decisive significance as regards the incidence of the atrophic skin changes.

When studying the frequency of non-palpable foot pulses in arteria dorsalis pedis and arteria tibialis posterior it was found that the women in the diabetic material lacked a palpable pulse in the arteria tibialis posterior to a much greater extent than the men and that among the women the frequency of non-palpable arteria tibialis posterior was considerably greater than the frequency of non-palpable arteria dorsalis pedis. This is probably due to anatomical differences in the sexes owing to an increased amount of subcutaneous fat or oedema. The arteria dorsalis pedis is, on account of its superficial location, more easily palpable and here, there was no difference between the sexes.

The frequency of non-palpable pulse in the arteria dorsalis pedis in patients with and without atrophic skin changes, is seen in Table 12. The total frequency of non-palpable arteria dorsalis pedis was 22 per cent. The frequency of non-palpable arteria dorsalis pedis in the group with atrophic skin changes was greater than in the group without such changes. The difference was probably

significant. ( $\chi^2 = 5.06$  df = 1  $0.025 > P > 0.01$ )

### Hypertension

The frequency of hypertension has been considered to be somewhat greater in diabetics than in non-diabetics. That this is the case with diabetics with nephropathy is unmistakable. That the frequency of hypertension is greater in diabetics without nephropathy in comparison with non-diabetics is more doubtful (Lundbaek 1953).

In a large material of apparently healthy people Master (1961) found that the systolic as well as the diastolic pressure rose with increasing age and that the pressure in women was higher than in men of the same age group. The increase of blood pressure culminated in men at the age of 65 years, whereas in women it continued to rise up to the age of 70 to 74. A diabetic material that is predominantly represented by elderly people reveals a higher frequency of hypertension when compared with a normal material without this predominant representation. Very often a diabetic material is predominantly represented by elderly women. It will then contain a

TABLE 12 The frequency of non-palpable pulse in arteria dorsalis pedis in diabetic with and without atrophic skin lesions

	With		Without		Total	
	Cases	%	Cases	%	Cases	%
Men	22/76	29	6/40	15	27/116	23
Women	13/42	31	17/98	16	29/140	21
Total	35/118	30	23/138	15	58/256	22



TABLE 13 *The frequency of hypertension in diabetic men and women with and without atrophic skin lesions*

	With		Without		Total	
	Cases	%	Cases	%	Cases	%
Men	19/78	24	6/42	14	25/120	21
Women	20/46	44	43/111	39	63/157	40
Total	39/124	31	49/153	32	88/277	32

greater frequency of women than men with hypertension if the same lower limit for hypertension is used for men as well as women

The frequency of hypertension in the whole material was 32 per cent of the men 21 per cent had hypertension and of the women 40 per cent. The frequency of hypertension in patients with and without atrophic skin lesions is seen in Table 13. There was no significant difference in this respect between patients with and without atrophic skin changes either as regards men or women (Men  $\chi^2 = 1.12$   $df = 1$   $0.3 > P > 0.2$  Yates's correction Women  $\chi^2 = 0.141$   $df = 1$   $0.8 > P > 0.7$  Yates's correction). On the other hand there was a significant difference in the frequency of hypertension between men and women ( $\chi^2 =$

11.44  $df = 1$   $P < 0.001$ ). The difference in frequency was most pronounced in the age group exceeding 60 years. Of the 123 patients exceeding 60 years of age 14 of 41 men (34 per cent) had hypertension and 47 of 82 women (57 per cent).

The frequency of hypertension in relation to age and diabetic duration is seen in Table 14. The frequency of hypertension rose with increasing age but on the other hand there was no rise in the frequency in relation to increasing diabetic duration as will be seen from the total frequency in the lowest row and the right column of the Table. Neither was there any difference in the frequency of hypertension between patients with less than and more than a 15 year diabetic duration ( $\chi^2 = 0.363$   $df = 1$   $0.6 > P > 0.5$  Yates's correction).

TABLE 14 *The frequency of hypertension in diabetic in relation to age and diabetic duration*

Duration Years	Age groups				Total	
	< 20	20-39	40-59	> 60		
	%	%	%	%		%
0-4	0/11	0/10	10/26	27/59	37/106	35
5-9	0/9	0/11	7/12	14/24	16/56	29
10-14	0/6	0/14	3/11	16/26	19/57	33
> 15	—	2/31	4/13	4/14	16/58	28
Total	0/26	2/66	19/62	61/123	88/277	32

### Other findings

Hereditary diabetic disposition was found in 46 per cent of the whole material the frequency being about the same for men and women. There was no significant difference in the incidence of hereditary diabetic disposition in patients with and without atrophic skin lesions ( $\chi^2$  0.959 df 1 0.6 >  $P > 0.5$ )

Varicose disease was found in 14 per cent — women 16 per cent, men 11 per cent. As regards the incidence of varices there was no significant difference between patients with and without atrophic skin changes ( $\chi^2$  0.771 df 1 0.4 >  $P > 0.5$  Yates's correction). Varicose ulcers were found in 9 patients.

Necrobiosis lipoidica was found in 9 patients — 2 men and 7 women.

Twelve patients had active or healed pulmonary tuberculosis, but tuberculous skin lesions were not diagnosed in any of the patients.

The majority of the patients were treated with insulin, or sulfonylureas and in only 9 per cent was diet alone considered to give satisfactory results.

Among the patients with atrophic skin lesions 26 per cent of the men had heavy work, 38 per cent had lighter work and 36 per cent had sedentary work. The majority of the women were housewives with light or moderate manual duties.

The group with divergent skin lesions consisted of 16 patients whose ages varied from 18 to 85 years — 10 men and 6 women. They were evenly distributed within all the age groups, the median age being 60 years.

The lesions consisted to a great extent of isolated pigmented spots without

atrophy. In two or three cases there was a more diffuse pigmentation and in some cases a few small excoriations with crusts were observed.

The patients were also evenly distributed within the groups with different diabetic duration. There were 4 patients in the group exceeding 15 years diabetic duration. In the group with less than 5 years duration there were 6 patients. In the latter group all the patients, with the exception of one were more than 40 years of age. As already seen a corresponding condition was found in the material with atrophic skin lesions. (Table 3.) The patients with atrophic skin changes and with a diabetic duration of less than 5 years were more than 40 years of age the majority more than 60 years.

Retinopathy was found in more than half of the patients with divergent skin changes, and neuropathy was found in 6 of the 16 patients.

Earlier in the presentation it was seen that the atrophic skin changes gradually subsided. Atrophy and pigmentation disappeared. In some cases there was, however in the later stages a remaining slight pigmentation without atrophy.

The similarity of the divergent skin lesions with older atrophic skin changes, and other similarities as regards age, diabetic duration and complications between patients with divergent skin changes and those with atrophic skin changes may probably denote that the former are an expression of older healed atrophic skin lesions and therefore may be included among them.

## THE HYPERTENSION MATERIAL

The occurrence of atrophic skin lesions of the described type in non-diabetics appears to be very rare. In a normal population it is hardly ever seen. A number of patients with hypertension were examined, the reason for this being that the arteriosclerotic vascular changes associated with hypertension might possibly give rise to similar atrophic skin lesions.

A material of 104 patients with hypertension as the principal disease were examined. The material consisted of 53 men and 51 women all of whom were admitted to the Medical Clinic in Umeå.

On the whole, the age distribution was comparable to that of the diabetic material although somewhat more evenly distributed and lacked the double curve peaks of the latter. The median age was somewhat lower in the hypertension material than in the diabetic one. The median age for men was 52.6 years and that for women was 53.1 years. Corresponding figures for the reported diabetic material was 50.0 and 60.3 years respectively. The youngest patient with hypertension was 19 and the oldest 77 years of age.

Manifest diabetes was not diagnosed in any of the patients. In 8 patients (8 per cent) there was a hereditary diabetic disposition. In the diabetic material the corresponding figure was 46 per cent.

Nine patients — 6 men and 3 women — had similar atrophic skin lesions on the legs to those observed in diabetics. The ages of these patients varied from 45 to 63 years and they had had hypertension from 3 to 17 years. Two of them had malignant hypertension. A diabetic

heredity was found in 1 patient.

In 7 of the 9 patients the skin lesions could not be differentiated from the diabetic ones. The two remaining patients had somewhat divergent changes, and only some single brownish pigmented skin lesion with insignificant atrophy was found.

In 6 of the 7 cases with typical lesions an oral glucose tolerance test was performed in a manner that will be described in Chapter V. Two patients had a diabetic curve that did not return to the fasting value three hours later. Four patients had a glucose tolerance curve that returned to the fasting value within 3 hours, but which rose maximally to more than 200 mg per cent (221—250 mg per cent). One year later one of these patients developed a manifest diabetes which confirmed the pathogenicity in the 3 other curves. The two patients with atypical skin lesions had glucose tolerance curves which returned to the fasting value within 3 hours and which did not exceed 200 mg per cent.

The patients with skin changes corresponding to those observed in diabetics had thus a diminished glucose tolerance and were assessed as latent diabetics. One of them later developed a manifest diabetes.

In view of the possibility of there being a traumatic origin to the atrophic skin changes a group of 50 active football players whose ages varied from 17 to 40 years, were examined during the current football season. As these players are subject to repeated leg injuries it was thought that they might reveal a high frequency of traumatic skin changes. No atrophic skin lesions of the described

type were found. Many of the players had superficial excoriations and some of them had diffuse pigmentations. A 70 year old man, a so-called old boy who had not taken an active part in the match, had atrophic skin lesions of the described type on the legs, but had also diabetes.

In a woman with systemic lupus erythematosus (SLE) and without signs of a manifest diabetes, brownish atrophic skin lesions were observed on the lower legs. However no glucose tolerance test was performed owing to her poor condition.

In connection with this finding an examination was made of a material of

patients with collagen diseases including SLE, dermatomyositis and polyarteritis nodosa with regard to atrophic skin lesions on the legs. Vascular changes have also been described in several of these diseases (cf Angervine 1961 Mackay and Burnet 1963) The material consisted of 30 patients. Only one patient more, a 40 year old man with SLE, had atrophic skin changes on the legs. He had, however a pathologic glucose tolerance curve and later developed a manifest diabetes.

## CHAPTER V

## ATROPHIC SKIN LESIONS IN THE LOWER EXTREMITIES OF PATIENTS WITHOUT MANIFEST DIABETES

One of the incentives to a systematic study of the question relating to the atrophic skin lesions in the lower extremities in diabetes was as already mentioned in the introduction the observation that these lesions could also occur in middle aged and old patients without glucosuria and that an oral glucose tolerance test in these cases often revealed a so-called latent diabetes. The observation denoted that the occurrence of these lesions was partly associated with a change of the glucose metabolism in a diabetic direction and partly could be an early and directly obvious sign of diabetes.

Those patients with the aforementioned skin lesions without glucosuria and an earlier diagnosed diabetes were subjected to examination with the oral glucose tolerance test.

The glucose tolerance test may be performed in many different ways (Conn 1940 Mosenthal and Berry 1950 Fajans and Conn 1954 Ikko and Luft 1957). In this investigation the oral glucose tolerance test was chosen. Statements as to where the limit goes between a normal and reduced glucose tolerance vary among different authors and *in* *inter alia* due to the choice of method for glucose determination and if the capillary or venous blood have been examined, and also the age of the control material (Jackson 1952 Chesrow and Bleyer

1954 Conn and Arbor 1958 Crombie and FitzGerald 1962 Oberdisse et al 1962). As a rule the glucose tolerance is interpreted on the basis of the blood sugar value 1.5 to 2 hours after the ingestion. According to another interpretation the blood sugar should have returned to the fasting value within a given time, usually from 2 to 2.5 hours. For old people the glucose tolerance curve is said to return within a period of 3 hours after the loading (Mosenthal and Berry 1950 Chesrow and Bleyer 1954 1957 Hecht et al 1961). As the glucose tolerance is thus diminished in elderly people, it is expedient when interpreting the glucose tolerance test to have a control material comparable in age.

## MATERIAL

The patient material with atrophic skin lesions, but without glucosuria or previously known diabetes was not obtained by a systematic study of a certain patient material and does not therefore permit conclusions concerning the frequency of this symptom. The material was obtained more or less, by incidental observations of these lesions in patients who were admitted to the Medical Clinic, and also as a result of the search for the lesions in patients examined in the Out Patient Clinic.

The material consisted of 17 patients whose ages ranged from 40 to 83 — median age 58 years, all of whom were admitted to the clinic at the time of examination. All of them had typical atrophic skin lesions on the lower parts of the legs, but without glucosuria or earlier diagnosed diabetes. Three patients had slight polynematosis corresponding to that found in diabetes. None of the patients had noticeable diabetic vascular changes such as retinopathy or nephropathy. Six of the patients had close relatives with diabetes. All the patients were examined with the oral glucose tolerance test.

The control material consisted of 46 inpatients comparable in age (40 to 78 years — median age 53) without diabetes and diabetic heredity. None of the patients was bedridden at the time of examination.

Of those patients with atrophic skin lesions 8 had diagnosed cardiovascular and 3 of these had a previously diagnosed cardiac infarction. None of the patients had collagen disease. The majority of the comparative material had slight arthritis and spondyloarthritis for which they were receiving treatment.

#### METHOD

The oral glucose tolerance test was performed after the patients were put on a rich carbohydrate diet (>300 g carbohydrate) for 3 days. On the morning following a 12 hours fast 100 g of glucose was ingested. Capillary blood for blood glucose assessment was taken partly before loading (fasting value) and partly every half hour after the ingestion

during a period of 4 hours. The blood sugar was assessed according to a modification of the glucose-oxidase method (Keston 1956 Teller 1956 Levin and Linde 1962). The blood sugar values obtained were plotted on a mm-square paper with the time along the abscissa and the blood sugar values along the ordinate. The resultant surface was determined planimetrically and the value obtained represented a measure of the glucose tolerance. The significance test was carried out according to Fisher's *t* formula for testing the difference between means (Guldford 1950).

Unfortunately the blood sugar method was altered during the investigation period by changing the precipitation of the blood protein which gave somewhat higher blood glucose values. The material was thus divided into two series (Series I and II) before and after the rearrangement. Series I included 10 patients with atrophic skin lesions and 19 patients as comparative material. Series II included 7 patients with atrophic skin changes and 27 patients as comparative material.

#### RESULTS

The mean curve for the control patients in both series is seen in Fig. 3. Both curves return to the fasting value within 3 hours. The curves rise maximally up to 152 and 159 mg per cent respectively.

Of the glucose tolerance curves for the 10 patients in Series I with atrophic skin lesions 6 had not returned to the fasting value three hours later. Of the 4 patients whose curves had returned to the fasting value within 3 hours, 2 of them had so-

called lag curves with a maximal rise to 200 mg per cent. Of the glucose tolerance curves for the 7 patients with atrophic skin changes in Series II all excepting one had not returned to the fasting value within 3 hours. The remaining patient had a lag-curve with a maximal peak at 215 mg per cent.

The mean value of the planimetrically estimated surface for the glucose tolerance curves in Series I was greater in patients with atrophic skin lesions in comparison with the controls. The difference was significant ( $t = 2.661$   $df = 27$

$0.02 > P > 0.01$ ). In Series II also the mean value of the planimetrically assessed surface was greater in patients with atrophic skin lesions when compared with the controls. The difference was significant ( $t = 2.214$   $df = 32$   $0.05 > P > 0.025$ ).

The patients with atrophic skin lesions and without glucosuria thus differed from a comparative material of corresponding age in that they had a statistically proved change of the oral glucose tolerance. The oral glucose tolerance had changed in a diabetic direction.

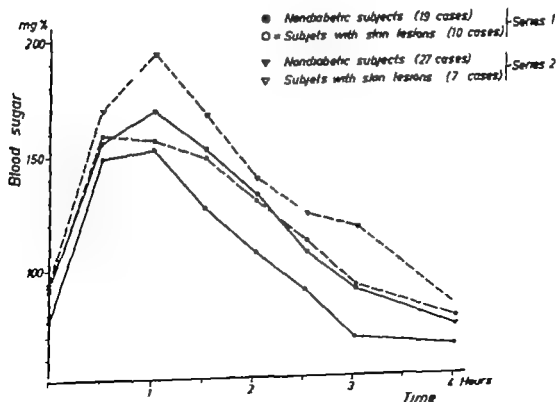


FIG 3 Oral glucose tolerance curve from patient with atrophic skin lesion and without known diabetes compared to non-diabetic control of the same age

## CHAPTER VI

### HISTOPATHOLOGICAL FINDINGS

Histopathological studies of the vascular lesions in the skin muscles and nerves in the lower extremities of diabetics were conducted by Goldenberg et al (1959). Without a previous knowledge of the clinical history they examined specimens from 152 amputations from the lower extremities (92 diabetics and 60 non-diabetics) with reference to the small vessels. Only those cases that showed a combination of endothelial hypertrophy and hyperplasia and PAS-stained structures in the walls of the small vessels were classified as «diabetic». Successive sections were stained with hematoxylin-eosine, periodic acid Schiff (PAS), colloidal iron of Hale modified by Rinehart and Abul-Haj (1951) and with Verhoeff-van Gieson. The Abul-Haj technique was used for separating arteriosclerotic structures from non-arteriosclerotic ones. There was only 2 per cent diagnostic error in the 152 specimens diagnosed as diabetic instead of non-diabetic. The digital artery was the largest in which the diabetic lesion could be distinguished. The «diabetic» vessel changes were found in 92 per cent of the diabetics, and in 1 per cent of the non-diabetics. They were found in 97 per cent of the vasa vasorum, in 73 per cent of the vasa nervorum and in 63 per cent of the skin vessels including the capillaries. In about half the cases there was no clinical evi-

dence of either retinopathy or nephropathy. The authors concluded that the lesions demonstrated in the small vessels of diabetics could be distinguished from arterio- and arteriosclerosis. Thus the diabetic lesions were characterized by endothelial proliferation in which were deposited PAS-positive fibrils in a reticulated pattern and not stained with colloidal iron (Abul-Haj). The most striking changes were found in the arterioles and capillaries.

A confirmatory study of the lower extremities of diabetics was conducted by Pedersen and Olsen (1962). They laid stress upon the often uneven and segmental distribution of the lesions in the small vessels.

Similar changes of the small vessels in the skin of the forearm were demonstrated by Handelsman et al (1962) in 8 of 12 diabetics of the juvenile and maturity-onset type without other clinical vascular changes and with diabetes of relatively recent onset. Of 6 patients with retinopathy and nephropathy all had distinctive lesions in the small vessels.

In an electron microscopic study on skin from the pulps of 4 diabetics Aagaard and Moe (1961) found the capillary wall much thicker when compared with the non-diabetic controls. The widening



was due to material similar in density and structure to the basement membrane

# MATERIAL

The material consisted of skin specimens from the lower parts of the legs in 11 patients with diabetes. In 4 patients the skin specimens were obtained at autopsy and were the same as those studied by microangiography. The remaining specimens consisted of skin biopsies. In all these cases the specimens con-

sisted of the previously described atrophic skin changes with adjacent normal skin.

The comparative material consisted of 5 patients. Four of them were autopsies. The remaining specimen was that of an amputated leg from a patient with pelvic sarcoma. The skin specimens from 3 of the patients were the same as those used for the microangiographic examination. Clinical data of the material is presented in Table 15.

TABLE 15 *The diabetic material and the non-diabetic control material used for histopathological examination of skin from the lower extremities*

			Diabetics			Diagnosed as diabetic
Patient		Age (Yr)	Duration of diabetes	Complications		
1	I. J.	female	32	23 yr	Rp np, neurop	+
2	H. A.	male	36	20 yr	Rp, np	—
3	N. L.	male	61	5 yr	Rp	+
4	E. M.	male	64	6 yr	Rp, np, neurop	+
5	S. L.	male	40	21 yr	Rp neurop	+
6	M. P.	male	40	15 yr	Rp np neurop	+
7	V. G.	male	48	Latent diabetes	—	+
8	A. H.	male	59	2 m.	—	—
9	L. N.	male	59	31 yr	Rp neurop	+
10	G. E.	male	63	13 yr	Rp neurop	+
11	E. B.	male	66	1 w	—	+

			Non-diabetic controls			Disease
Patient		Age (Y)				
1	B. E.	male	21	Acute myocarditis		—
2	S. E.	male	53	Malignant hypertension		—
3	F. L.	male	59	Cancer ventricle		—
4	E. L.	female	71	Acute pancreatitis		—
5	G. J.	male	37	Pelvic sarcoma		—

1—4 of the diabetics and 1—4 of the controls were autopsy specimens and 5—11 of the diabetics were biopsies. Control No. 5 was a specimen from an amputated lower extremity. Rp. denotes diabetic retinopathy, np. denotes diabetic nephropathy and neurop. denotes diabetic neuropathy.

## METHOD

The skin specimens were fixed in 10 per cent formalin and embedded in paraffin. In order to search for the vascular changes mentioned above successive sections from each paraffin block were stained with haematoxylin-eosine, periodic acid-Schiff, Ruchart and Abul-Haj (1951) colloidal iron staining and Gomori & Giesson staining. One of the blocks from each of the previously described microangiographic specimens was serially sectioned and stained in the same way. Specimens from the biopsy material were also stained with regard to the presence of hemosiderin (Berlin blue reaction) and melanin (Masson) in order to gain some idea of the type of pigment in the atrophic skin changes.

The slides were examined without a previous knowledge of the clinical history and then re-examined with the clinical key. It was assumed that the cases with diabetes would show a combination of endothelial proliferation and an increase of PAS-stained material especially in the capillaries and small vessels in the stratum papillare as pointed out by Handelman et al. (1962). They used a 4-graded scale for the alterations and found that the 3rd and 4th degrees of alteration were chiefly present in diabetics.

## RESULTS

All told there were two diagnostic errors in the specimens from the 11 diabetic patients classified as non-diabetic (Table 15). One was a 36 year old man with a diabetic duration of 20 years and with both retinopathy and nephropathy. The other was a newly discovered dia-

betes in a 59 year old man without clinical diabetic complications.

In the remaining cases the vascular changes were often pronounced. They appeared best in the PAS-staining. The most advanced changes were chiefly found in the superficial dermis — stratum papillare. The capillaries often showed thickened walls, sometimes with obliteration of the lumen. On the other hand, the large vessels in the deeper layers of the dermis did not show pronounced changes as often and consequently they were not as well correlated to the diabetic disease as the small vessels in the papillary layer of the dermis. The 5 patients in the comparative material were all with regard to the skin specimens, diagnosed as non-diabetics. The superficial vessels in the dermis were throughout without corresponding changes, or with only insignificant PAS-deposits in the walls.

When the slides from the two erroneously diagnosed diabetic patients were compared with the control specimens, after the clinical diagnosis was known, no definite difference was found between them as regards the vascular changes and consequently there was no reason to change the diagnosis.

The atrophic skin lesions consisted of atrophy of the epidermis sometimes with indicated hyperkeratosis. A similar but not so extensive atrophy of the epidermis could sometimes be seen in the skin outside the atrophic areas. In the dermis there was slight fibrosis with a change in the collagenous structure. In some cases the dermis showed edema with separation of collagen fibers which, in these

cases, was probably a question of early skin lesions.

The occurrence of vessels in the dermis varied widely and the vessel changes and degree of change did not deviate from that of the adjacent skin (Fig 15 p 73). Relatively normal vessels, especially in the papillary layer could be seen side by side with distinctly changed vessels (Fig 16 p 73). On some occasions a slight round cell infiltration was observed round the vessels. The Abul Haj stained sections were negative as regards the superficial vessels in the dermis indicating the absence of arteriosclerotic changes. The evaluation was complicated in that a number of structures in the dermis are normally coloured in the Abul-Haj staining. No definite changes were seen

in the subcutis. On certain occasions a slight increase of fibrous tissue was found.

The amount of melanin in the atrophic skin lesions could be increased within the basal cell layer of the epidermis, but similar changes were also seen in the adjacent skin and also in non-diabetic skin. In the dermis of the lesions there was often seen an insignificant quantity of hemosiderin.

The brown colour of the atrophic lesions was probably due to the melanin being more visible as a result of the atrophy of the epidermis and an increased quantity of hemosiderin. In connection with abundant vascularization this may be decisive for the colour (See page 46).

## CHAPTER VII

## MICROANGIOGRAPHIC STUDIES

With the aid of microangiography it is possible to visualize the blood vessels as far down as to the capillaries. In contact microangiography the object is placed in close contact with a fine-grained photographic emulsion during exposure. The object is reproduced in natural size on the microradiogram. The primary microradiogram is studied in the microscope or enlarged according to usual photographic procedures. The maximal degree of enlargement is inter alia dependent upon the geometric definition and the resolution of the photographic emulsion. Stereo-microangiography affords a better conception of the internal relation of the vessels (cf. Bellman 1953, Odén et al 1958).

According to current conceptions of the blood supply of the skin the arterial vessels from the subcutis form a plexus in the transition to the dermis. From here the vessels penetrate the dermis and form a new subpapillary network parallel to the surface of the skin, from which small vessels finally supply the papillaries in the shape of one or more capillary loops in each papilla. In a corresponding manner the veins form draining plexuses in the skin. A shunting of the blood may partly take place through coarse arteriovenous shunts and partly through so-called preferential channels (cf. Spalteholz 1927, Montagna 1962).

## METHOD

The investigation was carried out on autopsy material. The arteries and vena femoralis were cannulized immediately below the groin. The leg was filled with contrast by means of the artery catheter. The contrast consisted of Micropaque (Damanay & Co). The suspension was warmed to body temperature. Twenty per cent of Micropaque in isotonic saline followed by 20 per cent of Micropaque in 3 per cent of gelatin solution was injected. The injection was done rapidly with a 20 cc glass syringe. In order to obtain a better filling of contrast a tourniquet was placed around the leg immediately below the site of cannulization with a free passage through the catheters. After the contrast injection the skin was cooled in running water whereupon skin specimens 2-2 cm. in size were excised with the subcutis attached. The skin lesions had been previously marked with Indian ink on the skin surface. The excised specimens were mounted on a cork disc and fixed in 10 per cent of formalin and later embedded in paraffin.

For the microangiographic examination a Machlett X-ray therapy tube was used, Type AEG 50, with a 1 mm beryllium window and a wolfram anode. For the exposure 20 mA and 15 kV were used. The photographic emulsion was Kodak M. R. Plate which has a resolu-

ion of 500—1000 lines per mm. To avoid light exposure the plates were wrapped in 12 micron thick aluminium foil. The objects were stereo-micro-radiographed in toto and vertical to the skin surface. The specimens were then cut into 3—4 mm thick sections vertical to the skin surface. These sections were stereo-micro-radiographed vertically to the cut surface. This projection was found most suitable for the study of the superficial skin vessels.

Apart from the contrast in the paraffin-embedded specimens having decreased somewhat in diameter no difference was seen in the microangiogram before and after the embedding. At histological examination the contrast was found as far out as to the capillaries in the papillary layer of the skin.

## MATERIAL

The material consisted of skin from 4 diabetic patients 3 of whom had atrophic skin lesions on the legs. The ages of the patients ranged from 34 to 64 years all of whom had long-term diabetic complications. All told 17 skin specimens with atrophic skin lesions were examined and 5 specimens of clinically normal skin. As a comparative material the skin from 4 non-diabetic patients (20 to 52 years of age) was examined 3 of whom had chronic nephritis, hypertension and acute myocarditis respectively. The fourth specimen was that of an amputated leg from a patient with pelvic sarcoma. All together the comparative material consisted of 6 skin specimens.

## RESULTS

The microangiograms of skin from the non-diabetic revealed an abundance of vessels and the capillaries in the papillary layer of the dermis were also observed (Fig. 4 p. 69). As a rule the distribution of the vessels was not so schematic as previously considered and more often than not no demonstrated arterial plexuses were observed. No contrast deposits signifying extravasation of contrast were found.

The skin from the diabetic presented a different picture to that from the non-diabetic. The macroscopically macroscopic diabetic skin was rich in larger vessels in the lower parts of the dermis. The intermediate and superficial dermis was however very poor in vessels in comparison to the skin in the control material. The small vessels were irregular in shape and varied in calibre, and the capillaries in the papillary layer could seldom be observed. In 4 specimens there were some small spherical contrast deposits at different depths in the dermis attached to the small vessels. It was difficult to decide if they consisted of extravasal contrast or were of another nature e.g. microaneurysms.

As regards the vessel supply the atrophic skin lesions often presented a different picture to that of the adjacent skin. In 9 of the 17 skin lesions there was a greater abundance of small vessels in the dermis than in the nearby skin (Fig. 5 p. 69). A great number of small vessels and capillaries radiating from the larger and deeper vessels were often observed to spread fan-wise towards the surface of the skin. No interruption of the contrast in the larger vessels of the

dermis could be seen as a sign of an occlusion.

The investigation thus proved that a good half of the examined atrophic skin lesions in diabetics were richer in capillaries than in the adjacent skin. The

macroscopically intact diabetic skin especially in the superficial parts of the dermis was not so rich in vessels as the non-diabetic skin in the comparative material.

ion of 500—1 000 lines per mm. To avoid light exposure the plates were wrapped in 12 micron thick aluminium foil. The objects were stereo-micro-radiographed in toto and vertical to the skin surface. The specimens were then cut into 3—4 mm thick sections, vertical to the skin surface. These sections were stereo-micro-radiographed vertically to the cut surface. This projection was found most suitable for the study of the superficial skin vessels.

Apart from the contrast in the paraffin-embedded specimens having decreased somewhat in diameter no difference was seen in the microangiogram before and after the embedding. At histological examination the contrast was found as far out as to the capillaries in the papillary layer of the skin.

#### MATERIAL

The material consisted of skin from 4 diabetic patients, 3 of whom had atrophic skin lesions on the legs. The ages of the patients ranged from 34 to 64 years, all of whom had long term diabetic complications. All told 17 skin specimens with atrophic skin lesions were examined and 5 specimens of clinically normal skin. As a comparative material the skin from 4 non-diabetic patients (20 to 52 years of age) was examined, 3 of whom had chronic nephritis, hypertension and acute myocarditis respectively. The fourth specimen was that of an amputated leg from a patient with pelvic sarcoma. All together the comparative material consisted of 6 skin specimens.

#### RESULTS

The microangiograms of skin from the non-diabetics revealed an abundance of vessels and the capillaries in the papillary layer of the dermis were often observed (Fig. 4 p. 69). As a rule the distribution of the vessels was not so schematic as previously outlined and more often than not no demarcated arterial plexuses were observed. No contrast deposits signifying extravasation of contrast were found.

The skin from the diabetics presented a different picture to that from the non-diabetics. The macroscopically intact diabetic skin was rich in larger vessels in the lower parts of the dermis. The intermediate and superficial dermis was, however, very poor in vessels in comparison to the skin in the control material. The small vessels were irregular in shape and varied in calibre and the capillaries in the papillary layer could seldom be observed. In 4 specimens there were some small spherical contrast deposits at different depths in the dermis attached to the small vessels. It was difficult to decide if they consisted of extravascular contrast or were of another nature, e.g. microaneurysms.

As regards the vessel supply the atrophic skin lesions often presented a different picture to that of the adjacent skin. In 9 of the 17 skin lesions there was a greater abundance of small vessels in the dermis than in the nearby skin (Fig. 5 p. 69). A great number of small vessels and capillaries radiating from the larger and deeper vessels were often observed to spread fan-wise towards the surface of the skin. No interruption of the contrast in the larger vessels of the

the lower extremities (Roswit et al. 1953 Scott 1961). Moreover the disappearance rate in subcutaneous tissue is much slower than that of the skin (Wisham and Yalow 1952) and it is well known that skin has a greater number of blood vessels than subcutaneous tissue.

It may be assumed that some of the injected isotope e. g.  $\text{Na}^{24}$  is cleared by the lymph vessels. By measuring the radioactivity in the ductus thoracicus in the dog, Stone and Miller (1949) found that only about 1 per cent of  $\text{Na}^{24}$  locally injected in the muscle was carried away by lymphatic drainage, and that the predominating part was cleared by the blood vessels.

The slow disappearance of  $\text{I}^{131}$  labeled albumin from subcutaneous tissue in human beings, in contrast to the rapid disappearance of radioiodide from the same tissue indicated that the former substance might be cleared by lymphatic drainage. (Hollander et al. 1961)

Local disappearance studies on diabetes at rare Rues et al. (1960) found a decreased subcutaneous disappearance rate in diabetes as compared with non-diabetics. The low disappearance rate was said to be correlated to the diabetic duration and thus an expression of a general microangiopathy.

Theoretical technical as well as medical aspects of disappearance measurements have been extensively described by Odeblad and Westin (1959)

#### MATERIAL AND METHOD

An investigation was carried out with the aid of  $\text{I}^{131}$  (half life 8.1 d.) and  $\text{Na}^{24}$  (h.l. 15.1 h.) in diabetic patients with atrophic skin changes on the legs.

The isotope was intradermally injected in the atrophic skin lesion and, by way of comparison, in adjacent normal skin. In a similar manner a number of patients were studied with atrophic scars of a definite traumatic genesis. All the patients were hospitalized.

A scintillation counter (NaI (TI) crystal) with a pulse height analyzer was used for the measurements. From the analyzer the pulses were registered both by a scaler and a ratemeter with a pen recorder. The time constant for the rate meter was 0.5 seconds. The detector was surrounded by a lead shield of at least 4.5 cm in thickness, and was supplied with a cylindrical collimator 2.5 cm in diameter and 6.0 cm in length. The pulse height analyzer was adjusted to the 364 keV photopeak of  $\text{I}^{131}$  and the 1.38 MeV photopeak of  $\text{Na}^{24}$ . The width of the channel was about 60 keV. The background was about 100 counts per minute (cpm) and the injected activity about 1 microcurie gave 5–6,000 cpm.

In a special series of tracer tests the two isotopes were measured simultaneously by using two pulse height analyzers with ratemeters. In this series the channel widths of the two pulse height analyzers were adjusted to give about the same counting rates for  $\text{I}^{131}$  and  $\text{Na}^{24}$ . The chemical form for  $\text{I}^{131}$  was sodiumiodide and for  $\text{Na}^{24}$  sodiumchloride both being diluted in isotonic saline.

A micrometer syringe was used in order to inject small volumes. The accuracy of the volumes delivered was stated to be  $\pm 0.0005$  ml. One complete revolution of the micrometer head delivered a volume of 0.01 ml but the head was graded in fiftieths and the smallest vol-



# THE DISAPPEARANCE RATE OF RADIOACTIVE IODIDE ( $I^{131}$ ) AND SODIUM ( $Na^{24}$ ) IN THE ATROPHIC SKIN LESIONS

After a local injection of radioactive isotopes the disappearance of the isotope may be studied by in vivo measurement. Small radioactive ions may after intrasutal injection be expected to disappear preferably through diffusion and the disappearance rate may give elucidating information about the local circulation in the tissue.

The basis for this procedure was mathematically and experimentally analyzed by Kety (1948 1949). He used  $Na^{24}$  and postulated that the disappearance or clearance of the isotope from the site of injection followed a single exponential function. It was also suggested that the disappearance rate yielded a rough index of the effective circulation.

Disappearance studies have been carried out on different tissues such as the muscle, the subcutaneous tissue and the skin (Kety 1948, Elkin et al. 1948, Wisaham et al. 1951, Barron et al. 1951, Braithwaite et al. 1951, McGirt 1952, Hanser et al. 1961). Disappearance studies of muscle and subcutis have been carried out to examine the circulation in the lower extremities in cases of arterial disease (Walder 1953, Philipp and Ruf 1953, Pabst 1955).

A relatively good correlation has been found between the blood flow as judged by plethysmography and the disappea-

rance rate in muscle under different conditions (Rapaport et al. 1952).

No practical difference in the disappearance rate between  $Na^{24}$  and  $I^{131}$  has been found (Hyman et al. 1950, Bauer et al. 1953). For practical reasons  $I^{131}$  has often been preferable because of its longer half life.

Clearance studies on the skin have chiefly been carried out in the field of plastic surgery for the evaluation of the circulation in grafts (Barron et al. 1951, Braithwaite et al. 1951, Hanser et al. 1961).

Only a few studies on the conditions in dermatologic diseases have been carried out to gain some idea of the local circulation (Bettley and Fairburn 1957, Blocker et al. 1957).

It has been pointed out that the total circulation in the skin could not be measured by this method. This is due to the disappearance rate being dependent upon the capillary blood flow and not being influenced by the flow through the arterio-venous shunts or by-pass channels in the skin. The disappearance rate is practically an expression of the nutritive circulation (Kety 1953, Walder 1953). In agreement with this it has been established that skin areas rich in capillaries such as the face have a more rapid clearance rate than that found in areas poor in capillaries e.g. the abdomen and

the lower extremities (Roswin et al. 1953; Scott 1961). Moreover the disappearance rate in subcutaneous tissue is much slower than that of the skin (Winham and Yalow 1952) and it is well known that skin has a greater number of blood vessels than subcutaneous tissue.

It may be assumed that some of the injected isotope e.g.  $\text{Na}^{24}$  is cleared by the lymph vessels. By measuring the radioactivity in the ductus thoracicus in the dog, Stone and Miller (1949) found that only about 1 per cent of  $\text{Na}^{24}$  locally injected in the muscle was carried away by lymphatic drainage and that the predominating part was cleared by the blood vessels.

The slow disappearance of  $^{131}\text{I}$ -labelled albumin from subcutaneous tissue in human beings, in contrast to the rapid disappearance of radioiodide from the same tissue, indicated that the former substance might be cleared by lymphatic drainage. (Hollander et al. 1961)

Local disappearance studies on diabetes are rare. Rys et al. (1960) found a decreased subcutaneous disappearance rate in diabetes as compared with non-diabetics. The low disappearance rate was said to be correlated to the diabetic duration and thus an expression of a general microangiopathy.

Theoretical, technical as well as medical aspects of disappearance measurements have been extensively described by Odéblad and Westin (1959)

#### MATERIAL AND METHOD

An investigation was carried out with the aid of  $^{131}\text{I}$  (half-life 8.1 d.) and  $\text{Na}^{24}$  (h.l. 15.1 h.) in diabetic patients with atrophic skin changes on the legs.

The isotope was intradermally injected in the atrophic skin lesion and, by way of comparison, in adjacent normal skin. In a similar manner a number of patients were studied with atrophic scars of a definite traumatic genesis. All the patients were hospitalized.

A scintillation counter ( $\text{NaI}$  (Tl) crystal) with a pulse height analyzer was used for the measurements. From the analyzer the pulses were registered both by a scaler and a ratemeter with a pen recorder. The time constant for the ratemeter was 0.5 seconds. The detector was surrounded by a lead shield of at least 4.5 cm in thickness, and was supplied with a cylindrical collimator 2.5 cm in diameter and 6.0 cm in length. The pulse height analyzer was adjusted to the 364 keV photopeak of  $^{131}\text{I}$  and the 1.38 MeV photopeak of  $\text{Na}^{24}$ . The width of the channel was about 60 keV. The background was about 100 counts per minute (cpm) and the injected activity about 1 microcurie gave 5–6,000 cpm.

In a special series of tracer tests the two isotopes were measured simultaneously by using two pulse height analyzers with ratemeters. In this series the channel widths of the two pulse height analyzers were adjusted to give about the same counting rates for  $^{131}\text{I}$  and  $\text{Na}^{24}$ . The chemical form for  $^{131}\text{I}$  was sodiumiodide and for  $\text{Na}^{24}$  sodiumchloride both being diluted in isotonic saline.

A micrometer syringe was used in order to inject small volumes. The accuracy of the volumes delivered was stated to be  $\pm 0.00005$  ml. One complete revolution of the micrometer head delivered a volume of 0.01 ml but the head was graded in fifteenths and the smallest vol-

ume delivered thus corresponded to one 0.0002 ml. The needle a fine gauge steel needle No. 26 was cemented to the syringe to eliminate leakage. The syringe was siliconized to avoid back flow leakage.

Somewhat varying volumes were used for the intradermal injections during the examinations. But however the same volume was used on each separate occasion. The injected volume varied from 0.03 to 0.002 ml and contained about 1 microcurie of the radioactive material. The larger volumes were, above all, conditioned by the low activity of the  $\text{Na}^{24}$  on arrival from the pile.

The examination was performed with the patient in a recumbent position. The investigation commenced after a 30 minute rest during which the background activity was registered. The temperature in the investigation room was practically constant 22–23°C. Blair et al (1959, 1960) has shown that there is no appreciable vasoconstrictor or vasodilator tone in the skin vessels of the leg at this temperature and when the patient is comfortably warm.

Intradermal injections were given alternating in the atrophic skin lesion and in the normal skin. The aperture of the collimator was placed a few mm above the site of injection. The measurement was started about one minute after the injection.

The activity was registered with the aid of the recorder and as an extra check by counting the number of impulses for a period of one minute every other minute. As a rule, the measurements were completed 15 minutes later. In a corresponding manner a new intradermal in-

jection was given adjacent to the first point of measurement. Prior to this injection the body background was measured at the point for the new one. The distance between the points of measurement was about 10 cm.

When the measurement values had been corrected for the background they were plotted against time on a semi-logarithmic paper where these values were placed in accordance with the logarithmic scale and the time according to the linear scale. The disappearance constant, the  $k$  value, was then calculated. The  $k$  value was obtained according to the formula

$$k = \frac{\log C_1 - \log C_2}{0.4343 (t_2 - t_1)}$$

Where  $C_1$  and  $C_2$  are the counts per minute at  $t_1$  and  $t_2$  respectively (Kety 1949).

A simpler way of obtaining the  $k$  value is to decide the time to decrease the activity by half, the biological half time ( $T_{1/2}$ ). The  $k$  value may then be obtained according to the formula

$$k = \frac{0.693}{T_{1/2}}$$

where 0.693 is the natural logarithm for 2.

For the significance test of the paired series Fisher's  $t$  formula for testing the difference between correlated pairs of means was used (Guilford 1950).

For the significance test of the non-paired series Fisher's  $u$  formula was used for the difference between uncorrelated pairs of means (Guilford 1950).

#### PREPARATORY STUDIES

In order to ascertain if the somewhat varying volumes used had any effect on

the disappearance rate of the isotope, tests were carried out on 3 patients. In each of them the intradermal disappearance rate in normal skin of the leg was assessed at 0.002, 0.01 and 0.03 ml. intradermally injected  $I^{131}$ . No difference in the disappearance rate was recorded in the three patients. The mean k-values were 0.111 (0.082 - 0.133), 0.122 (0.091 - 0.157) 0.115 (0.091 - 0.139) for the three different volumes respectively.

In order to ascertain if there was any difference in the disappearance rate between  $I^{131}$  and  $Na^{24}$  a mixed isotope solution with both isotopes was injected. The pulse height analyzer was so adjusted that the counting rate was about the same for both isotopes. The test was carried out on 5 patients, 3 of whom had diabetes. The disappearance rates for both isotopes were almost identical (Table 16).

In order to ascertain if there was any difference in the disappearance rate between two adjacent deposits of intradermally injected  $I^{131}$  15 patients were examined, 2 of whom had diabetes with durations of 2 and 5 years and the others were non-diabetics. All of them were

healthy without signs of cardiovascular disease. The injections were given 8 to 10 cm. from each other in a proximal-distal direction on the front of the lower part of leg, and alternately the proximal and distal point were first injected and measured. The results are given in Table 17. There was no statistical difference in the disappearance rate between the two points measured. ( $t = 0.282$   $df = 14$   $0.8 > P > 0.7$ )

## RESULTS

In a series of 17 patients with diabetes and with atrophic skin lesions on the lower legs the disappearance rate of  $I^{131}$  was assessed partly in the atrophic skin lesion and partly in the intact adjacent skin (Table 18). The ages and diabetic duration of the patients are given in the Table. The mean k-value of the intact skin was 0.071. There was no difference in the disappearance rate for patients below or above the median age in the group. Nor was there any difference in the disappearance rate between patients who had a diabetic duration of less or more than 15 years.

TABLE 16. *Disappearance rate of  $I^{131}$  and  $Na^{24}$  in common intradermal deposits in the normal skin of the leg.*

				$I^{131}$		$Na^{24}$	
				T 1/2	k-value	T 1/2	k-value
1	T	K.	20 yr male	12.2	0.057	12.2	0.057
2	C	W	23 yr male	8.6	0.081	8.6	0.081
3	A	H.	37 yr female	22.0	0.031	21.0	0.033
4	A.	E.	38 yr male	24.0	0.029	23.0	0.030
5	E	T	63 yr female	27.0	0.026	26.0	0.027
Mean				18.8	0.045	18.2	0.046

\* Diabetics with duration of diabetes 15, 31 and 12 years respectively

ume delivered thus corresponded to one 0.0002 ml. The needle a fine gauge steel needle No. 26 was cemented to the syringe to eliminate leakage. The syringe was siliconized to avoid back flow leakage.

Somewhat varying volumes were used for the intradermal injections during the examinations. But however the same volume was used on each separate occasion. The injected volume varied from 0.03 to 0.002 ml and contained about 1 microcurie of the radioactive material. The larger volumes were, above all, conditioned by the low activity of the  $\text{Na}^{24}$  on arrival from the pile.

The examination was performed with the patient in a recumbent position. The investigation commenced after a 30 minute rest during which the background activity was registered. The temperature in the investigation room was practically constant 22–23° C. Blair *et al* (1959, 1960) has shown that there is no appreciable vasoconstrictor or vasodilator tone in the skin vessels of the leg at this temperature and when the patient is comfortably warm.

Intradermal injections were given alternating in the atrophic skin lesion and in the normal skin. The aperture of the collimator was placed a few mm above the site of injection. The measurement was started about one minute after the injection.

The activity was registered with the aid of the recorder and as an extra check, by counting the number of impulses for a period of one minute every other minute. As a rule the measurements were completed 15 minutes later. In a corresponding manner a new intradermal in-

jection was given adjacent to the first point of measurement. Prior to this injection the body background was measured at the point for the new one. The distance between the points of measurement was about 10 cm.

When the measurement values had been corrected for the background they were plotted against time on a semi-logarithmic paper where these values were placed in accordance with the logarithmic scale and the time according to the linear scale. The disappearance constant, the  $k$  value, was then calculated. The  $k$  value was obtained according to the formula

$$k = \frac{\log C_1 - \log C_2}{0.4343 (t_2 - t_1)}$$

Where  $C_1$  and  $C_2$  are the counts per minute at  $t_1$  and  $t_2$  respectively (Kery 1949).

A simpler way of obtaining the  $k$  value is to decide the time to decrease the activity by half, the biological half time ( $T_{1/2}$ ). The  $k$  value may then be obtained according to the formula

$$k = \frac{0.693}{T_{1/2}}$$

where 0.693 is the natural logarithm for 2.

For the significance test of the paired series Fisher's  $t$  formula for testing the difference between correlated pairs of means was used (Guilford 1950).

For the significance test of the non-paired series Fisher's  $t$  formula was used for the difference between uncorrelated pairs of means (Guilford 1950).

#### PREPARATORY STUDIES

In order to ascertain if the somewhat varying volumes used had any effect on

On the other hand, there was a distinct difference in the disappearance rate between the atrophic skin lesions and the adjacent intact skin i. e. the disappearance was quicker in the atrophic skin changes in comparison with that of the normal skin. The difference was significant. ( $t = 7.616$   $df = 16$   $P < 0.001$ )

A similar condition arose in a study with  $Na^{24}$  in a corresponding manner in 6 diabetics with atrophic skin changes. The mean  $k$  value was 0.073 ( $T \frac{1}{2}$  9.5 mm.) in the atrophic skin lesions against 0.047 ( $T \frac{1}{2}$  14.9 mm.) in the intact skin. The difference was significant ( $t = 2.635$   $df = 5$   $0.05 > P > 0.025$ ).

In a series of patients with old, established traumatic scars in the lower extremities a comparison was made of the disappearance rate in the scar with that in the adjacent skin by the same method. It is well known that the tissue in an old scar is poor in vessels, and consequently the disappearance rate in the scar should be slower than in the adjacent normal skin.

The series consisted of 21 patients without diabetes, their ages ranging from 19 to 61 years. All the patients were able to state how and when the trauma occurred the appearance of the scar corresponded to their statements. It was chiefly a matter of scars from slashes, bruises and post-operative scars. The ages of the scars varied from 2 to 46 years. All of them were bleached and atrophic. Every patient was examined with  $I^{131}$  as well as with  $Na^{24}$  (Table 19). As was to be expected, a lower disappearance rate of  $I^{131}$  as well as  $Na^{24}$  was found in the scars when compared with the adjacent skin. The difference was

statistically significant ( $I^{131} : t = 4.894$   $df = 20$   $P < 0.001$   $Na^{24} : t = 3.439$   $df = 20$   $P < 0.005$ ).

Thus old traumatic scars differ from atrophic skin lesions in diabetics in that the disappearance of  $I^{131}$  and  $Na^{24}$  in the scar is significantly slower and in the atrophic skin lesion significantly quicker than in the adjacent intact skin.

In new traumatic scars there is an abundance of vessels in the granulation tissue. Weiber (1959) revealed that with the isotope technique applied to animals the vascularization of the healing wound progressed successively during the first 5 days and then gradually regressed. About a week later the disappearance rate for the scar approached that of the adjacent skin.

Traumatic scars in diabetics of about the same age as the atrophic skin lesions were also compared. A small series of 5 diabetic patients with relatively newly formed traumatic scars (1 to 2 years old) comparable in age to the atrophic skin changes, were tested with  $I^{131}$  in a corresponding manner (Table 20). The disappearance rate in the scar was also lower than in the intact skin. The mean  $k$  value in the scar was 0.071 and in the adjacent skin 0.088. If the disappearance rate in these scars was compared with that of the atrophic skin lesions (Table 18) the mean  $k$ -value 0.071 and 0.106 respectively. The difference was significant. ( $t = 2.657$   $df = 20$   $0.02 > P > 0.01$ )

As regards the disappearance of  $I^{131}$  traumatic scars in diabetics differ from atrophic skin changes of about the same age. This suggests that the atrophic skin lesions are not traumatic but spontaneous. Since the comparison of the ages

TABLE 17 *Disappearance rate of intradermally injected ITH in two adjacent deposits in the normal skin of the leg*

				Proximal		Distal	
				T $\frac{1}{2}$	k-value	T $\frac{1}{2}$	k-value
1	K S	17 yr	female.	6.1	0.114	5.5	0.126
2	B. G	18 yr	male.	6.0	0.116	6.6	0.105
3	L. A.	19 yr	male.	9.2	0.075	10.5	0.066
4	H. A.	27 yr	male.	8.6	0.081	9.0	0.077
5	A. J	28 yr	female.	5.0	0.139	5.8	0.119
6	K. H	33 yr	female.	5.2	0.133	6.2	0.112
7	G S	38 yr	male.	4.3	0.161	10.6	0.065
8	L. B	38 yr	female.	8.2	0.085	8.7	0.080
9	G E	49 yr	male.	6.6	0.105	6.0	0.116
10	I S	49 yr	female	5.3	0.151	5.3	0.131
11	A. A.	52 yr	female.	12.1	0.057	8.5	0.082
12	F G	50 yr	male	10.9	0.064	10.1	0.069
13	V L.	56 yr	male.	6.6	0.105	8.4	0.083
14	O L.	59 yr	male.	6.0	0.116	5.6	0.124
15	C. G	59 yr	male.	9.8	0.071	8.6	0.081
Mean				7.3	0.104	7.7	0.096

) Diabetics with duration of diabetes 5 and 2 years respectively

TABLE 18. *Disappearance rate of intradermally injected ITH in atrophic skin lesions and in adjacent intact skin on the legs of diabetics*

				Intact skin		Atrophic skin lesion	
				T $\frac{1}{2}$	k-value	T $\frac{1}{2}$	k-value
1	H. V	17 yr	Dur 11 yr male	13.0	0.053	5.3	0.131
2	H. J	24 yr	Dur 14 yr male	7.0	0.099	5.3	0.131
3	B. V	33 yr	Dur 15 yr male	15.5	0.046	8.7	0.080
4	K. A.	35 yr	Dur 20 yr male.	8.2	0.085	6.0	0.116
5	R. K.	41 yr	Dur 25 yr female.	6.2	0.112	4.8	0.144
6	M. P	42 yr	Dur 15 yr male.	10.2	0.068	7.6	0.091
7	I. J.	44 yr	Dur 22 yr male	8.5	0.082	8.5	0.082
8	Y. G	44 yr	Dur 17 yr male	7.7	0.090	4.7	0.147
9	A. S	50 yr	Dur 5 yr male	13.5	0.051	9.0	0.077
10	E. N	53 yr	Dur 32 yr male.	10.5	0.066	5.0	0.139
11	R. M.	57 yr	Dur 11 yr male.	12.9	0.054	6.6	0.105
12	E. T	60 yr	Dur 13 yr female	8.2	0.085	6.3	0.110
13	M. J	60 yr	Dur 10 yr male	9.0	0.077	6.8	0.102
14	B. B	68 yr	Dur 12 yr male	9.2	0.075	5.0	0.139
15	R. H.	69 yr	Dur 17 yr male.	14.5	0.048	10.9	0.064
16	O. O	74 yr	Dur 1 yr male	12.5	0.055	9.5	0.073
17	F. F	74 yr	Dur 9 yr male	15.0	0.053	9.0	0.077
Mean				10.6	0.071	7.0	0.106

TABLE 20. *Disappearance of intradermally injected I<sup>125</sup> in traumatic scars 1 to 2 year old and in the adjacent intact skin in the leg of diabetic*

	Traumatic scar		Normal skin		Trauma
	T 1/2	k-value	T 1/2	k-value	
1. E. E. 32 y. Dur. 14 yr female.	14.5	0.048	7.0	0.099	Traum. scar 1 yr
2. E. M. 26 yr. Dur. 8 y male.	9.0	0.077	10.6	0.061	Op. scar 2 yr
3. B. H. 41 yr Dur. 15 y female.	8.3	0.084	8.2	0.086	Op. scar 1 yr
4. K. B. 60 yr Dur. 23 yr male.	9.1	0.076	5.7	0.122	Op. scar 2 yr
5. H. J. 72 yr Dur. 8 yr male.	10.0	0.069	9.7	0.071	Traum. scar 1 yr.
Mean	10.2	0.071	8.2	0.088	

\* ) N = clearance.

of the traumatic scars and the atrophic skin changes are subject to a certain amount of uncertainty the Author considers this conclusion scarcely conclusive.

In order to ascertain if there was any difference in the disappearance rate of I<sup>125</sup> between intact diabetic and nondiabetic skin the values for the disappearance rate from the intact skin of the 21 patients with traumatic scars (Table 19) were compared with the values from the intact skin of the 17 patients with

atrophic skin lesions (Table 18). In both series the patients were comparable as regards age. The mean k value for the non-diabetic skin was 0.105 and for diabetic skin 0.071. The difference was significant ( $t = 3.141$  df = 36  $0.005 > P > 0.001$ ).

Thus the disappearance for I<sup>125</sup> is slower in diabetic skin than in non-diabetic skin. As previously mentioned there was no difference in the disappearance rate in relation to diabetic duration.



TABLE 19 D appearance rate of  $\text{Iu}$  and  $\text{Na}^{26}$  in old traumatic scars and in adjacent normal skin in the legs of non-diabetics

	$\text{Iu}$		$\text{Na}^{26}$		Trauma
	Normal skin $T \frac{1}{2}$ k value	Traumatic scar $T \frac{1}{2}$ k-value	Normal skin $T \frac{1}{2}$ k-value	Traumatic scar $T \frac{1}{2}$ k value	
1 I S 19 yr male	8.0	0.087	10.8	0.064	Op. scar 3 yr
2 H B 20 yr male	5.8	0.119	7.0	0.099	Traum. scar 8 yr
3 L K 22 yr male	9.2	0.075	8.4	0.083	Traum. scar 2 yr
4 A W 25 yr male	6.7	0.103	7.7	0.090	Op. scar 10 yr
5 L T 26 yr male	3.5	0.198	10.7	0.045	Traum. scar 15 yr
6 J F 30 yr male	7.0	0.099	9.8	0.071	Traum. scar 18 yr
7 F W 31 yr male	4.8	0.144	8.3	0.084	Traum. scar 16 yr
8 K J 32 yr male	8.0	0.087	16.7	0.042	Burn. scar 22 yr
9 H S 34 yr male	5.3	0.131	11.4	0.061	Traum. scar 24 yr
10 K M 42 yr male	5.0	0.159	8.4	0.083	Traum. scar 30 yr
11 S J 45 yr male	8.8	0.079	10.2	0.068	Traum. scar 29 yr
12 H M 48 yr male	10.0	0.069	16.0	0.043	Traum. scar 27 yr
13 S J 49 yr male	5.5	0.126	7.7	0.090	Traum. scar 35 yr
14 H L 52 yr male	8.9	0.078	13.9	0.050	Op. scar 24 yr
15 H L 52 yr male	8.9	0.078	10.0	0.069	Traum. scar 35 yr
16 H O 55 yr male	8.7	0.080	9.2	0.075	Traum. scar 18 yr
17 B K 56 yr male	4.1	0.169	4.9	0.141	Traum. scar 46 yr
18 B H 56 yr male	7.2	0.096	12.0	0.058	Op. scar 3 yr
19 H W 59 yr male	7.9	0.088	9.6	0.072	Op. scar 11 yr
20 M L 61 yr male	10.5	0.066	10.3	0.051	Traum. scar 30 yr
21 T J 63 yr male	7.2	0.096	17.5	0.040	Traum. scar 45 yr
Mean	7.2	0.105	10.5	0.072	
			8.3	0.091	
			11.6	0.071	

2. Two patients with systemic lupus erythematosus (SLE) were also examined. Both of them had atrophic skin lesions of the same appearance as those seen in diabetes. One of them had also diabetes of one year's duration. In one of these patients the specimens were taken immediately after death.

3. As a control normal skin from the lower extremities was examined in two healthy persons without diabetes or cardiovascular disease.

## RESULTS

1. In the controls a very weak, diffuse fluorescence was observed in the epithelial cells of the epidermis. The specificity test revealed no significant decrease and the finding was considered unspecific. Otherwise no certain fluorescence was seen.

2. In the non-controls three different types of fluorescence were observed

a. Fluorescence of the epithelial cells in the epidermis. This was seen partly as a diffuse yellowish-green colour similar to that in the normal cases, and partly as a pronounced fluorescence around the cells. The latter fluorescence was generally more distinct in the basal cell layer of the epidermis. It was observed in the specimens from the 2 patients with SLE and in 4 of the diabetes. It diminished but was not completely eliminated at the control tests and was considered probably specific. (Fig. 1-III p. 74). It was not observed in the controls.

b. The specimens from all the diabetics and the two patients with SLE showed a diffuse partly speckled but rather weak fluorescence of the dermis immedi-

ately under the basal cell layer of the epidermis. The fluorescence was entirely inhibited at the control tests. It could not with certainty be correlated to PAS-positive structures.

These two types of fluorescence were not only observed in the atrophic skin lesions, but also in the normal adjacent skin. They were considered probably specific. Corresponding fluorescence was not found in the controls.

c. Fluorescence of small vessels in the dermis immediately under the basal cell layer of the epidermis. This fluorescence was entirely eliminated at the control test and could definitely be correlated to thickened, strongly PAS-positive capillary walls. (Fig. IV p. 75). This fluorescence was considered specific. It was observed in the specimens from the two patients with SLE and all the 6 patients with diabetes. It was seen in the atrophic skin lesions as well as in adjacent normal skin from the two diabetic patients examined. It was not seen in the controls.

## DISCUSSION

The investigation appears to speak in favour of the fact that bound gammaglobulin may occur in the thickened PAS-positive capillary walls in the intact skin and in the atrophic skin lesions on the lower extremities of diabetics. The condition was analogous in two cases with SLE. Burnham et al (1963) and Cormane (1964) report the incidence of bound gammaglobulin in the basal membranes of the epidermis in skin lesions from inter alia discoid lupus and systemic lupus erythematosus. The fluo-

## CHAPTER IX

## AN INVESTIGATION WITH THE FLUORESCENT ANTIBODY TECHNIQUE ON THE OCCURRENCE OF GAMMAGLOBULIN IN SKIN FROM THE LOWER EXTREMITIES OF DIABETICS

By

*Olle Larsson & Hans Melin*

It has previously been established that the PAS-positive glomerular and vascular changes in diabetic nephropathy contain gammaglobulin (Odin and Törnblom 1959 Freedman et al 1960) and probably complement (Freedman and Markowitz 1962)

It was thought therefore that it would be of interest to investigate the atrophic skin lesions on the lower extremities in diabetics (Melin 1962 1964) with the fluorescent antibody technique to establish the presence of bound gamma globulin. In these skin lesions as well as in the intact skin of diabetics PAS-positive vascular changes have also been observed.

## METHOD

Skin was taken with punch biopsy from the lower extremities. The specimens were immediately frozen and subsequently cut into 5 micron thick sections in cryostat and fixed in cold acetone after air-drying.

The fluorescent staining was carried out according to Coon's direct method (cf Mellors 1959). The specimens were stained with fluorescent rabbit antihuman gammaglobulin serum for 30 minutes at room temperature. The speci-

ficity of the staining was checked partly by some specimens being pre-treated for 15 minutes with non-conjugated antihuman gammaglobulin serum and partly by some specimens being exposed to gammaglobulin and fluorescent antihuman gammaglobulin serum at the same time.

Only if an obvious diminishing of the fluorescence could be registered was the result assessed specific.

Rabbit antihuman gammaglobulin serum conjugated with fluorescein isothiocyanate absorbed once with monkey kidney and twice with calf liver was used (Serum FS 363 received by Professor Astrid Fagraeus-Wallbom Statens Bakteriologiska Laboratorium Stockholm Sweden).

At least 4 specimens from each biopsy were stained, and equally as many for control. In 2 cases PAS-staining was performed on every other section of the skin specimen.

## PATIENT MATERIAL

1 Six diabetics with atrophic skin lesions on the lower extremities were examined. In 2 of them the specimens were also taken from clinically normal skin a few cm from the lesions.

## CHAPTER X

### DISCUSSION

The described atrophic skin lesions in diabetes are, according to the foregoing presentation, almost completely found in the lower extremities. They occur preferably only in diabetes. The group of patients with these skin lesions and without glucosuria or an earlier known diabetes, revealed, according to the oral glucose tolerance test, a change of the glucose metabolism in a diabetic direction. In patients with juvenile onset diabetes these skin lesions were observed only in those with a relatively long diabetic duration. In patients with maturity onset diabetes the lesions occurred after a shorter diabetic duration. The patients with atrophic skin lesions but without glucosuria were all middle aged or elderly people. The connection between the occurrence of the atrophic skin lesions and the incidence of so-called long-term diabetic complications of the vascular type was pronounced.

The aforementioned facts and the trophic nature of the lesions led to the presumption that they were of vascular origin. Thus the subsequent investigation concentrated upon the search for a local arterial occlusion being the cause of the lesions.

The microangiographic investigation revealed no local occlusion. Instead it showed that the dermis below the lesions usually displayed an increased vasculariza-

tion when compared with the adjacent normal skin. The established increase of the disappearance rate of a locally injected isotope ( $^{131}\text{I}$  and  $\text{Na}^{24}$ ) in the atrophic skin lesions compared to the nearby intact skin suggests an increased local circulation in the lesions.

There is naturally the possibility of the atrophic lesions having a traumatic origin. Localization in the lower parts of the legs and the dominant occurrence in males may support such an assumption. It may, however, be pointed out that only a fourth of the males with atrophic skin lesions had heavy manual work. A third had light work such as sedentary duties and so on. In the group of active football players without diabetes none of them had corresponding atrophic skin lesions.

A comparison of the disappearance of an isotope ( $^{131}\text{I}$ ) injected into these skin changes with the disappearance in proved traumatic scars on the lower legs from non-diabetic patients, showed that the isotope vanished considerably slower from the traumatic scars. This observation does not justify any definite conclusion being drawn in view of the origin of the atrophic lesions. The traumatic scars were older than the atrophic skin lesions.

The atrophic skin lesions were also different as regards the disappearance of

rescent antibody technique was also used here. No specific fluorescence was observed by these authors in the skin from normal persons. Cormane (1964) also observed a corresponding but weaker fluorescence in apparent normal skin in a few cases with SLE. This fluorescence was similar to that found in this investigation in the basal cell layer of the epidermis in diabetics (see 2 a.)

Specific fluorescence in the small vessels in the skin correlated to thickened PAS-positive capillaries does not appear to have been described previously either in SLE, diabetes or other disorders.

#### CONCLUSION

With the aid of the fluorescent antibody technique for the demonstration of human gammaglobulin in the skin from diabetics with atrophic skin changes the Authors have been able to demonstrate specific fluorescence of the thickened PAS-positive walls of the vessels in the dermis immediately under the basal cell layer of the epidermis.

In 4 of 6 diabetic patients fluorescence was also observed around the cells in the basal cell layer of the epidermis.

This was considered probably specific. The latter fluorescence has certain similarities with that observed by other investigators in inter alia discoid lupus and SLE. It was not only observed in the atrophic skin lesions on the lower extremities, but also in the apparently normal skin from diabetics. The fluorescence in SLE has similarly been described not only in skin lesions, but also in clinically intact skin. Corresponding fluorescence was not observed in the examination of skin from normal patients.

#### REFERENCES

- Burnham, T. K., Neblett, T. R. & Fine, G. *J. Invest. Derm.* 41: 451 1963
- Cormane, R. H.: *Lancet* 1: 534 1964
- Freedman, P. Peters J. M. & Kark, R. M. *Arch. intern. Med.* 105: 524 1960
- Freedman, P. & Markowitz, S.: *J. Clin. Invest.* 41: 328 1962.
- Melin, H. *Nord. Med.* 67: 661 1962
- Melin H. *Acta med. scand. Suppl.* 423 1964
- Mellors, R. C. *Analytical Cytology* 2nd ed. New York, McGraw-Hill Book Company Inc., 1959
- Müller-Eberhard, H. J. Odén, L. & Törnblom, N. *Nord. Med.* 61: 813 1959
- Odén, L. & Törnblom, N. *Acta Soc. Med. upsalen.* 64: 313 1959

## CHAPTER X

## DISCUSSION

The described atrophic skin lesions in diabetes are, according to the foregoing presentation, almost completely found in the lower extremities. They occur preferably only in diabetics. The group of patients with these skin lesions and without glycosuria, or an earlier known diabetes, revealed, according to the oral glucose tolerance test, a change of the glucose metabolism in a diabetic direction. In patients with juvenile onset diabetes these skin lesions were observed only in those with a relatively long diabetic duration. In patients with maturity onset diabetes the lesions occurred after a shorter diabetic duration. The patients with atrophic skin lesions but without glycosuria were all middle aged or elderly people. The connection between the occurrence of the atrophic skin lesions and the incidence of so-called long-term diabetic complications of the vascular type was pronounced.

The aforementioned facts and the atrophic nature of the lesions led to the presumption that they were of vascular origin. Thus the subsequent investigation concentrated upon the search for a local arterial occlusion being the cause of the lesions.

The macroangiographic investigation revealed no local occlusion. Instead it showed that the dermis below the lesions usually displayed an increased vasculariz-

ation when compared with the adjacent normal skin. The established increase of the disappearance rate of a locally injected isotope ( $^{131}\text{I}$  and  $\text{Na}^{24}$ ) in the atrophic skin lesions compared to the nearby intact skin suggests an increased local circulation in the lesions.

There is naturally the possibility of the atrophic lesions having a traumatic origin. Localization in the lower parts of the legs and the dominant occurrence in males may support such an assumption. It may however be pointed out that only a fourth of the males with atrophic skin lesions had heavy manual work. A third had light work such as sedentary duties and so on. In the group of active football players without diabetes none of them had corresponding atrophic skin lesions.

A comparison of the disappearance of an isotope ( $^{131}\text{I}$ ) injected into these skin changes with the disappearance in proved traumatic scars on the lower legs from non-diabetic patients, showed that the isotope vanished considerably slower from the traumatic scars. This observation does not justify any definite conclusion being drawn in view of the origin of the atrophic lesions. The traumatic scars were older than the atrophic skin lesions.

The atrophic skin lesions were also different as regards the disappearance of

<sup>1131</sup> from proved traumatic scars of about the same age in diabetics. The disappearance was significantly slower in the traumatic scars. It should however be noted that there is some uncertainty as regards the congruity in age between the atrophic skin lesions and the traumatic scars. Conformity between the two compared groups of diabetic patients concerning age at the onset of diabetes and diabetic duration has not been proved either. Furthermore, it is possible that a difference concerning the type of probable trauma may be of importance. The traumatic scars were *inter alia* caused by operative measures.

The fact that the disappearance of a locally injected isotope is slower even in these traumatic scars in diabetics than in the atrophic skin lesions does not in the opinion of the Author support the notion that the lesions are of traumatic origin but that they occur spontaneously.

In one case it was possible to assert definitely that the atrophic skin lesions had occurred spontaneously. The primary lesions were of a haemorrhagic nature and were neither tender nor irritable. They subsequently developed into typical atrophic skin lesions.

With regard to the absence of local clinical signs of infection and the histological picture it is improbable that the atrophic skin changes are caused by a local infection. Among the patients with juvenile diabetes it was seen that these changes only occurred in those who had had diabetes for a relatively long time — a fact that also excludes an infectious origin.

At the histological examination of the skin of diabetics with atrophic skin les-

ions it was observed that there were changes of the small vessels both in areas corresponding to the skin lesions and in the adjacent skin of the type described by Goldenberg *et al* (1959).

A comparison of the disappearance of <sup>1131</sup> in clinically normal skin on the legs of diabetics with the disappearance of <sup>1131</sup> in corresponding skin areas of non-diabetics showed that the disappearance was slower in diabetics. It is possible that there is some connection between the two described findings. Diabetic skin with changes of the small vessels and with a reduced circulation may be more vulnerable than normal skin. It is also possible that diabetic skin reacts in another way than normal skin to a trauma. The fact that an isotope disappears quicker from the atrophic skin lesions than from the aforementioned traumatic scars in diabetics with those reservations already made does not suggest that such is the case.

At the examination of the skin from diabetics with atrophic skin lesions with Coon's immunofluorescent technique concerning the occurrence of gammaglobulin fluorescence was observed partly in the capillary walls immediately below the epidermis and partly in the basal cell layer of the latter. The latter fluorescence was similar to that which authors have described in discoid and systemic lupus erythematosus. These fluorescence phenomena were observed not only in the atrophic skin changes, but also in the apparently normal skin of diabetics. Fluorescence in systematic lupus erythematosus has also been described not only in obviously changed skin areas, but also in normal skin. Corresponding fluores-

cence was not observed in the skin from the controls.

Organ involvements in systemic lupus erythematosus are generally considered to be due to immunological changes. The presence of gammaglobulin in the skin of diabetes with a localization similar to that in systemic lupus erythematosus warrants the presumption that the atrophic skin lesions are due to immunological changes. However this question must remain unanswered.

The basis for the assumption that the atrophic skin lesions are spontaneous and possibly conditioned by immunological changes has already been mentioned. The fact that they are nearly always found in the lower extremities obviously does not suggest a spontaneous origin. In this

connection it should be pointed out that a non-traumatic lesion such as erythema nodosum is chiefly localized in the lower extremities.

The more common occurrence of atrophic skin lesions in diabetic males than in diabetic females is difficult to explain. It should, however be borne in mind that e. g. polyarteritis nodosa occurs more frequently in males and lupus erythematosus and erythema nodosum is more common in females.

The atrophic skin lesions in diabetes should be considered a common and typical long-term diabetic complication. They have a characteristic appearance and are easily observed. Owing to their localization they are accessible for further detailed studies.



## SUMMARY

A survey is presented of the so-called long term diabetic vascular changes and of skin lesions associated with diabetes. A previously unknown skin lesion in diabetes is described. It consists of small rounded brownish atrophic and circumscribed lesions localized in the lower extremities. They appear isolated, or in groups or are sometimes of a linear pattern. It is shown that these skin changes disappear after one or more years, and that in the meanwhile new ones appear the picture as a whole remaining unchanged. More often than not the patients are not conscious of the lesions. Some patients consider them to be, insofar that they have noticed them, an expression of trauma. On the other hand some patients assert that they arose spontaneously. In one case it was possible to state definitely that the changes had occurred spontaneously.

A diabetic material of 277 patients, all of them more than 15 years of age were examined as regards the occurrence of the aforementioned skin lesions and so-called diabetic complications. The skin changes arose in 65 per cent of the males and in 29 per cent of the females. The incidence of the lesions was correlated to the diabetic duration and increased with the latter. The lesions occurred earlier in maturity onset diabetes than in juvenile onset ones. The atrophic skin

lesions were correlated to the incidence of long-term diabetic vascular complications such as retinopathy and nephropathy. The skin lesions were also correlated to neuropathy and to arterial disease of the lower extremities. On the other hand they were not correlated to the incidence of hypertension.

The occurrence of atrophic skin lesions of the described type in non-diabetics is very rare. A material of 104 patients with hypertension comparable in age, but without diagnosed diabetes, were examined to see if the arteriosclerotic vascular changes in this disease could possibly give rise to such lesions. Only 7 patients had skin lesions similar to those described. The oral glucose tolerance test showed that 6 of these patients had a diabetic tolerance curve.

A group of 17 patients with atrophic skin lesions congruent to those described but without glucosuria or earlier known diabetes were examined with the oral glucose tolerance test. They had a statistically proved change of the glucose tolerance in a diabetic direction as compared to a control material of a similar age.

In 2 patients with systemic lupus erythematosus skin lesions in the lower extremities were discovered which were congruent to the diabetic. One of these patients had a diabetic glucose tolerance

curve. In a further 28 patients with severe collagen disease no corresponding skin lesions were found.

At the histological examination of the skin lesions atrophy of the epidermis and sometimes slight fibrosis of the dermis were demonstrated. Pigment-stainings revealed no definite increase of the melanin content, and in a few cases an inconsiderable quantity of hemosiderin was found. Moreover PAS-positive vascular changes of the type previously described by other authors were observed, partly in the skin lesions and partly in the apparently intact skin.

A microangiographic study of autopsy material from diabetes with atrophic skin lesions showed that the dermis corresponding to the changes was often more abundantly vascularized than the adjacent intact skin.

Disappearance studies with a locally injected isotope ( $^{131}\text{I}$  and  $\text{Na}^{24}$ ) showed that there was a significantly quicker disappearance of the isotope in the atrophic skin lesions than in the nearby intact skin. In proven traumatic scars on the lower extremities of non-diabetics the disappearance was slower in the scars than in the neighbouring skin. In diabetes with traumatic scars of about the same age as the skin lesions the dis-

appearance of  $^{131}\text{I}$  was also slower in the scars than in the adjacent skin. The difference in the disappearance rate between the latter scars and the skin lesions was statistically significant. The importance of these observations is discussed. They are considered to speak in favour of the atrophic skin lesions not being of traumatic origin and that they occur spontaneously.

A fluorescence-microscopic examination of skin from diabetes with atrophic lesions with anti-human gammaglobulin revealed fluorescence partly in the capillary walls immediately under the epidermis, and partly in the basal cell layer of the epidermis. The latter fluorescence was similar to that demonstrated by former investigators in skin from patients with discoid and systemic lupus erythematosus. As in systemic lupus erythematosus this fluorescence was not only observed in the skin lesions, but also in apparently intact skin. The possibility of the atrophic skin lesions in diabetes being due to changed immunologic conditions is discussed.

The atrophic skin lesions in diabetes are a common and long-term diabetic complication which, as regards their site are easily accessible for further detailed studies.

To all those who in various ways contributed to the completion of this investigation I take this opportunity of expressing my thanks. I am very much indebted to Professor Lars-Göran Larsson for his valuable help and for permission to use the isotope equipment in

his department, and to Mr Lars Jonsson for his generous aid in electro-technical matters. To Professor Carl-Martin Fehrer I am deeply indebted for his guidance and constructive criticism in the histological examination. Thanks are also due to Professor Sög Bergström for kindly

placing the micro-angiographic apparatus at my disposal and to Docent Bo Odén whose skilful aid enabled me to become conversant with the micro-angiographic technique. I am very much obliged to Professor Lina Hollström for his good advice and criticism concerning dermatological problems. Thanks are also due to Mrs Margit Leonardsson for her indefatigable help with the patients. To the Staffs of the Departments of Ophthalmology and Clinical

Chemistry I would like to express my thanks for their valuable assistance. Docent Gunnar Borg and Wilhelm van der Linden generously gave me statistic advice. Valuable help was received from Farbenwerke Hoechst, Frankfurt. The investigation was supported by grants from the Medical Faculty of the Umeå University, Nordisk Insulinfond, Copenhagen and Långmanska kulturfonden, Stockholm.

## REFERENCES

- Aspöcker, U. & Moe, H. *Diabetes* 10: 253 1961.
- Aspöcker, U. *Neurovascular Examinations on the Lower Extremities in Young Diabetics, with Special References to the Autoimmune Neuropathy* Copenhagen, C. H. Hagerberg Bogtrykkeri 1962.
- Angervall, L., Dorevall, G. & Lehman, K.-E. *Acta med. scand.* 169 339 1961.
- Angervall, M. D. The Pathogenesis of Polyarteritis. In Mills, L. C. & Meyer, J. H. ed.: *Inflammation and Disease of Connective Tissue* Philadelphia, W. B. Saunders, 1961.
- Ashton, N. *Brit. J. Ophthalmol.* 33 407 1949.
- Ashton, N. *Brit. J. Ophthalmol.* 33 189 1951.
- Ballaerby, A. J. & Loevenstam, A. T. *Ophthalmol. Soc. U.K.* 63 95 1943.
- Ballaerby, A. J. & Loevenstam, A. T. *Brit. J. Ophthalmol.* 28 193 1944.
- Ballaerby, A. J. *Arch. Ophthalmol.* 33 97 1945.
- Birky, F. R. *Acta med. scand. suppl.* 304 1955.
- Barton, J. M., Veall, N. & Arnott, D. C. *Br. J. plast. Surg.* 4 16, 1951.
- Bauer, H. K., Cassen, B., Yountcheff, E. & Shoop, L. *Amer. J. med. Sci.* 225 374 1953.
- Beck, K., Hansen, E., Loretzen, S. E. & Lundbeck, K. *Diabetes* 9 441 1960.
- Bell, E. T. *Arch. Path.* 49 469 1950.
- Bell, E. T. *Arch. Path.* 53 444 1952.
- Bell, E. T. *Diabetes* 2 376, 1953.
- Bell, E. T. *Amer. J. clin. Path.* 28 27 1957.
- Bellman, S. *Acta radiol. suppl.* 102, 1953.
- Bergstrand, A. & Bockx, H. *Lab. Invest.* 6 293 1957.
- Bettley, F. R. & Fairburn, E. A. *Acta dermatol. venerol. Proc. 11th Internat. Congress Derm.* 601 1957.
- Buchoff, A. *Schweiz. med. Wochs.* 89 519 1959.
- Blair, B. A., Glover, W. E. & Roddie, I. C. *J. Physiol. (Lond.)* 149 19 P 1959.
- Blair, B. A., Glover, W. E. & Roddie, I. C. *J. Physiol. (Lond.)* 153 232, 1960.
- Blocker, T. G., Jr., Lewis, S. R., Perry, J. L., Tomhouch, W. T. & Lyon, W. L. *Ann. Surg.* 141 630, 1957.
- Blumenthal, H. T., Alex, M. & Goldenberg, S. *Arch. Path.* 70 13 1960.
- Bradley, R. P. & Bryfolge, J. W. *Amer. J. Med.* 20 207 1956.
- Brauhwiler, P., Farmer, F. T. & Herbert, F. L. *Brit. J. plast. Surg.* 4 34, 1951.
- Bryfolge, J. W. & Bradley, R. P. *Diabetes* 6 159 1957.
- Chenow, E. J. & Bleyer, J. M. *Geriatrics* 9 276, 1954.
- Chenow, E. J. & Bleyer, J. M. *Geriatrics* 12 171 1957.
- Clawson, B. J. & Bell, E. T. *Arch. Path.* 48 105 1949.
- Coom, J. W. *Amer. J. med. Sci.* 199 555 1940.
- Coom, J. W. & Arbor, A. *Diabetes* 7 347 1958.
- Crombie, D. L. & Fox-Gerald, M. G. *Br. med. J.* 1 497 1962.
- Dahlberg, G., Jorpes, E., Kallner, S. & Lichtenstein, A. *Acta med. scand. suppl.* 122, 1947.
- Dryog, A., Dobson, H. L. & Brennan, J. C. *Ann. Intern. Med.* 54 672, 1961.
- Düzel, J. *Circulation* 70-277 1954.
- Düzel, J. *The Conjunctival Vessels in Diabetes Mellitus* Copenhagen, Munksgaard, 1962.
- Dodd, H. & Cockett, F. B. *The Pathology and Surgery of the Veins of the Lower Limb* London, Livingstone, 1956.
- Dolger, H. *J. Amer. med. Ass.* 134 1298, 1947.
- Dey, T. J. & Hines, E. A. *J. Amer. intern. Med.* 14 1893, 1943.

placing the micro-angiographic apparatus at my disposal, and to Docent Bo Odén whose skilful aid enabled me to become conversant with the micro-angiographic technique I am very much obliged to Professor Einar Holmström for his good advice and criticism concerning dermatological problems. Thanks are also due to Mrs Margit Leonardsson for her indefatigable help with the patients. To the Staffs of the Departments of Ophthalmology and Clinical

Chemistry I would like to express my thanks for their valuable assistance, Docent Gunnar Borg and Wilhelm van der Linden generously gave me status as *ad hoc*. Valuable help was received from Farbenwerke Hoechst, Frankfurt. The investigation was supported by grants from the Medical Faculty of the Umeå University, Nordisk Insulinfond, Copenhagen and Långmanaka kulturfonden, Stockholm.

- McGee E. M. *Clin. Sci.* 11 91 1952.
- MacKinnon Royal London Ophthalmic Hosp. Report 9 112, 1877 C. in Joslin, E. P. Root, H. F. White, P. & Marble, A. ed. *The Treatment of Diabetes Mellitus*. 10th ed Philadelphia, Lea & Febiger 1919
- McKinnon, J. F. A. *Amer. J. Path.* 24 643 1941
- Meibow R. B. Meibow S. J. Pollack, H. Bookman, J. J. & Oserman, K. *Amer. J. Med.* 11 322, 1953
- McLennan, R. C. *Analytical Cytology* 2nd ed New York. Mc Graw-Hill Book Comp 1959
- Melin, H. *Nord. Med.* 67 661 1962
- Mendlowitz, H. Grossman, E. B. & Alpert, S. *Amer. J. Med.* 11 316 1953
- Montagu, W. *The Structure and Function of Skin* 2nd ed. New York, Academic Press, 1962.
- Monschall, H. O. & Berry E. *Ann intern Med.* 33 1271 1950.
- Murkesson, J. *Acta med scand.* 138 94, 1950
- Neuberg Trans. Ophthal Soc. United Kingdom 8 119 1888 C. in Joslin, E. P. Root, H. F. White, P. & Marble, A. ed. *The Treatment of Diabetes Mellitus* 10th ed Philadelphia, Lea & Febiger 1919
- Oakley W. Cammell, R. C. P. & Maron, M. M. *Brit. med. J.* 22 953 1946
- Oberdorfer, K. Black, H. & Hutter K. A. *Klin. Wochr.* 40 446, 1962
- Odeblad, E. & Wesson, B. *Acta radiol suppl* 173 1959
- Odén, B. Bellman, S. & Fries, B. *Br. J. Radiol.* 31 70, 1958
- Odén, L. & Torabloom, N. *Acta Soc. Med. Upsalica* 64 313 1959
- Oppenheim, M. *Zbl. Haut- u. Geschl. Kr.* 32 179 1929
- Paby, H. W. *Über die Anwendung von radioaktivem Jod zur Untersuchung der peripheren Durchblutung und ihrer pharmakologischen Beeinflussung* In Fellinger K. & Vetter H. ed. *Radioaktive Isotope in Klinik und Forschung* Bd. 33 München-Berlin, Urban & Schwarzenberg, 1955
- Pedersen, J. & Olsen, S. *Acta med. scand.* 171 151 1962.
- Pillsbury D. M., Shelley W. B. & Kligman, A. M. *Dermatology Philadelphia*, W. B. Saunders Comp., 1957
- Philipp, K. & Raf F. *Strahlentherapie* 90 553 1953
- Prud'Homme, J. *Un. med. Can.* 89 616 1960.
- Pyke, D. A. & Roberts, D. S. C. *Acta med. scand.* 163 489 1959
- Rapaport, S. I., Saul, A., Hyman, C. & Morton, M. E. *Circulation* 1 194 1952.
- Ries, W. Schuster L., Senge, A. & Wegner H. *Klin. Wochr.* 38 681 1960.
- Rinehart, J. F. & Abol-Haj, S. H. *Arch. Path.* 52 189 1951
- Rodriguez, R. & Root, H. F. *New Engl. J. Med.* 238 391 1948.
- Rollins, T. G. & Winkelmann, R. A. *Arch. Derm.* 82 537 1960.
- Root, H. F. *The Nervous System and Diabetes* In Joslin, E. P. Root, H. F. White, P. & Marble A. ed. *The Treatment of Diabetes Mellitus*. 10th ed Philadelphia, Lea & Febiger 1919
- Rovnit, B. Wabham, L. H. & Sorrentino, J. *Amer. J. Roentgenol.* 69 980, 1953
- Rundles, R. W. *Medicine (Baltimore)* 24 111 1945
- Sabour M. & McDonald, M. K. & Robson, J. S. *Diabetes* 11 291 1962
- Salon, R. *Med. Klinik* 1 256 1928
- Scott, A. T. *Ann. N. Y. Acad. Sci.* 100 111 1961
- Seigrist, K. *Acta med. scand. suppl.* 325 1957
- Späthholz, W. *Blutgef. aus der Haut*. In Jadassohn, J. ed. *Handbuch der Haut- und Geschlechtskrankheiten*. Erster Band. Erster Teil. Berlin, J. Springer 1927
- Sorenson, I. *Acta med. scand. suppl.* 394, 1963
- Stone, J. W. & Miller W. B. *Proc. Soc. exp. Biol. (NY)* 71 129 1949
- Sutton, R. L. *Diseases of the Skin*. 11th ed. S. Louis, C. V. Mosby Comp. 1956.
- Teller J. D. *Abstr. of Papers*. 130th Meeting. Amer. Chem. Soc. 69 C, 1956
- Urbach, E. *Arch. Derm. Syph. (Berl.)* 166, 273 1952.
- Urbach, E. *J. Amer. med. Ass.* 129 439 1945
- Walker D. N. *Clin. Sci.* 22 153 1953.

- Elkin D C, Cooper F W, Rohrer R H, Miller W B, Shea P C. & Dennis E W: Surg. Gynec. Obstet. 87 1 1948
- Fagerberg, S E. Acta med. scand. suppl. 345 1959
- Fajans, S S & Conn J W. Diabetes 3 296 1954
- Farquhar M G, Hopper J & Moon H D. Amer. J. Path. 35 721 1959
- Fitzpatrick, T B. Dermatologic Lesions and Diseases associated with Diabetes. In Wilkins, R. H. ed. Diabetes. New York, P B Hoeber 1960.
- Foley W T & Wright I S. Color Atlas and Management of Vascular Disease. New York, Appleton-Century-Crofts, 1959
- Goldenberg, S, Alex M. & Blumenthal H. T.: Diabetes 3 98 1958
- Goldenberg, S, Alex, M., Joshi R. A. & Blumenthal, H. T.: Diabetes 8 261 1959
- Goodman J, Baumol S, Frank I L, Marcus, L. J. & Wassermann, S.: The Diabetic Neuropathies. Springfield Illinois C. C. Thomas, 1955
- Goodof I I. Ann intern. Med. 22 373 1945
- Gusfield J P. Fundamental Statistics in Psychology and Education 2nd ed. New York Mc Graw Hill Book Comp., 1950
- Guy W B. Penn med. J. 54 1052 1951
- Girde, A. & Angelberg, E. Nord Med. 70 1252 1963
- Handelman M B, Morrone T G & Ghisman B: Arch intern. Med. 110 108 1962
- Harner W H, Tauxe W N, Owen, C. A. & Lippcomb P R. Surg. Gynec. Obstet. 112 625 1961
- Hanum S. Acta ophthalm. (Abh) suppl. 10 1959
- Hare, P. J. Brit. J. Derm. 67 365 1955
- Hecht, A. Weisenfeld S & Goldner M. G. Metabolism 10 712, 1961
- Hollander W, Reilly P, Burrows B A. J. clin. Invest. 40 222 1961
- Hyman, C, Rapaport S. J. & Poldno R. Amer. J. Physiol. 163 722 1950
- Ikkos, D. & Lof, R. Acta endocr. (Abh) 25 312 1957
- Jackson, W I U. Brit. med. J. 71 690, 1952
- Jordan W. Arch. intern. Med. 57 307 1936
- Jordan, E. P, Root, H. P, White, P. & Marble A. The Treatment of Diabetes Mellitus. 10th ed. Philadelphia, Lea & Febiger 1959
- Keston, A.: Abstr. of Papers 129th Meeting Amer. Chem. Soc. 31 C, 1956
- Kety S S. Amer. J. med. Sci. 215 352, 1948
- Kety S. S.: Amer. Heart J. 38 321 1949
- Kety S S.: Med. Sci. Publ. 3 128 1953
- Kummelstiel P & Wilson, C.: Amer. J. Path. 12 83 1936
- Kurtz O. Arch. Augenheilk. 110: 284 1937
- Kurtz, H., Hurwitz, L. J.: Ginsberg, S. M. & McDowell, F.: Arch. Neurol. (Chic.) 4 51 1961
- Lax, H. & Feinberg, A. W.: Circulation 20 1126 1959
- Lever W F.: Histopathology of the Skin 3rd ed. Philadelphia J. B. Lippincott Comp., 1961
- Levin, K. & Linde, S. Svenska Läk-tidn. 39 3016 1962
- Liebow I M, Hellerstein, H. A. & Miller M. Amer. J. Med. 18 438 1955
- Liss J R., Magiday M., Galloway I. & Hart, J. F.: J. Amer. med. Ass. 120 192 1942
- Locke S, Lawrence D G & Legg, M. A. Amer. J. Med. 34 773 1963
- Lundbaek, K. Ugeskr. Laeg. 111 1349 1949
- Lundbaek K. Long-term Diabetes. Copenhagen, Munksgaard, 1953
- Lundbaek K. Late Developments in Long-term Diabetic Vascular Disease. In ed. Diabetes Mellitus. III Kongress der Internationalen Diabetes Federation, Dusseldorf 1958 Stuttgart, G. Thieme, 1959
- Lundbaek, K. Medicinsk Årbok 1960—1961 Stockholm, A.B. Nordiska Bokhandeln 1960
- Mackay I R. & Burnet F. M.: Autoimmune Diseases. Springfield Illinois, C. C. Thomas, 1963
- Marble, A. Infections in Diabetes. In Joslin, E. P, Root, H. P, White P. & Marble A. ed. The Treatment of Diabetes Mellitus 10th ed. Philadelphia, Lea & Febiger 1959
- Marron, M. M. Brain 76 194 1953
- Mayer A. M. & Lamer P. P. Blood Pressure Elevation in the Elderly. I. Brent, A. N. & Meyer J. H. ed.: Hypertension. Recent Advances. Philadelphia Lea & Febiger 1961

- McGill E. M. *Clin. Sci.* 11 91 1932.
- MacKenzie Royal London Ophthalmic Hosp Report 9 152, 1877. Cit. in Joslin, E. P. Root, H. F. White, P. & Marble, A. ed. *The Treatment of Diabetes Mellitus*. 10th ed. Philadelphia, Lea & Febiger 1959.
- McLennan, J. F. A. *Amer J Path* 24 643 1943.
- Meghous R. & Meghous S. J. Pollack, H. Bookman, J. J. & Oserman, A. *Amer J Med* 15 122, 1953.
- McNair, R. C. *Analytical Cytology* 2nd ed. New York, McGraw-Hill Book Comp 1959.
- Melin, H. *Nord. Med.* 67 661 1962.
- Mendowitz, M. Grossman, E. B. & Alpert, S. *Amer J Med* 15 316, 1953.
- Montagna, W. *The Structure and Function of Skin*. 2nd ed. New York, Academic Press, 1962.
- Moorehead, H. O. & Berry E. *Ann. intern Med* 33 1175 1950.
- Murphy, J. *Acta med. scand.* 138 94 1950.
- Nutrition Trans Ophthal Soc United Kingdom 8 159 1951. Cit. in Joslin, E. P. Root, H. F. White, P. & Marble, A. ed. *The Treatment of Diabetes Mellitus*. 10th ed. Philadelphia, Lea & Febiger 1959.
- Oakley W. Catterall, R. C. F. & Martin, M. *M. Brit med J* 22 933 1956.
- Oberwieser K. Blask, H. & Huter K. A. *Nim. Wochr* 40 446, 1962.
- Odeblad, E. & Westin, B. *Acta radiol suppl* 173, 1959.
- Oden, B. Bellman, S. & Frick, B. *Brit J Radiol* 11 70, 1958.
- Oden, L. & Tornblom, N. *Acta Soc Med. Scand* 64 313 1959.
- Oppenheimer, M. *Zbl. Haut- u. Geschl.-Kr* 32 179 1929.
- Pabst, H. W. *Über die Anwendung des radioaktiven Jod zur Untersuchung der peripheren Durchblutung und ihrer pharmakologischen Beeinflussung*. I. Fellner K. & Vetter H. ed. *Radioaktive Isotope in Klinik und Forschung*. Bd 33. München-Berlin, Urban & Schwarzenberg, 1955.
- Pedersen, J. & Olsen, S. *Acta med. scand.* 171 551, 1962.
- Pillsbury D. M. Shelley W. B. & Kligman, A. M. *Dermatology Philadelphia*, W. B. Saunders Comp 1957.
- Philipp, K. & Ruf F. *Strahlentherapie* 90 553 1953.
- Prod'Homme, J. *Un. med. Can.* 29 616 1960.
- Price, D. A. & Roberts, D. S. C. *Acta med. scand.* 163 489 1959.
- Rapaport, S. L., Saul, A. Hyman, C. & Morton, M. E. *Circula* 105 594 1952.
- Ries, W. Schuster L., Seige, K. & Wegner H. *Klin. Wochr* 38 681 1960.
- Rothbart, J. F. & Abul-Haj, S. K. *Arch. Path.* 12 189 1951.
- Rodriguez, R. & Root, H. F. *New Engl. J Med* 238 391 1948.
- Rollins, T. G. & Winkelman, R. K. *Arch. Derm.* 82 557 1960.
- Root, H. F. *The Nervous System and Diabetes*. In Joslin, E. P. Root, H. F. White, P. & Marble A. ed. *The Treatment of Diabetes Mellitus*. 10th ed. Philadelphia Lea & Febiger 1959.
- Rosen, B. Wulzen, L. H. & Sorrentino, J. *Amer J. Roentgenol.* 69 980, 1953.
- Rundlet, R. W. *Medicine (Baltimore)* 24 111 1945.
- Sabour, M. S. McDonald, M. K. & Rabson, J. S. *Diabetes* 11 291 1962.
- Sahn, R. *Med. Klin.* 1 256 1928.
- Scott, A. T. *Ann. Sc. Johns. Hosp. Derm. Soc. (Lond)* 46 22, 1961.
- Sigroth, A. *Acta med. scand. suppl.* 325 1957.
- Spalteholz, W. *Blutgefäße der Haut*. In Jadassohn, J. ed. *Handbuch der Haut- und Geschlechtskrankheiten*. Erster Band. Erster Teil. Berlin, J. Springer 1927.
- Steinert, I. *Acta med. scand. suppl.* 394 1963.
- Stone, P. W. & Miller W. B. *Proc. Soc. exp. Biol. (NY)* 71 529 1949.
- Sutton, R. *The Diseases of the Skin*. 11th ed. St. Louis, C. V. Mosby Comp., 1956.
- Teller J. D. *Abstract of Papers*. 130th Meeting. Amer. Chem. Soc. 69 C, 1916.
- Urbach, E. *Arch. Derm. Syph. (Berl.)* 166 273 1932.
- Urbach, E. *J. Amer. med. Ass.* 129 459 1945.
- Walder D. N. *Clin. Sc.* 12 153, 1953.



- Elkin D C, Cooper F W, Rohrer R H, Miller W B, Shea, P C. & Dennis, E W: *Surg. Gynec. Obstet.* 87 1 1948
- Fagerberg, S E. *Acta med. scand. suppl.* 345 1959
- Fajans, S S & Conn J W. *Diabetes J* 296 1954
- Farquhar M G, Hopper J & Moon, H D. *Amer J Path.* 35 721 1959
- Fitzpatrick, T B: *Dermatologic Lesions and Diseases associated with Diabetes*. In Williams, R. H. ed. *Diabetes*. New York P B Hoeber 1960
- Foley W T & Wright I S: *Color Atlas and Management of Vascular Disease*. New York Appleton-Century Crofts, 1959
- Goldenberg, S. Alex, M. & Blumenthal, H T: *Diabetes* 7 98 1958
- Goldenberg, S., Alex, M., Joshi, R. A. & Blumenthal H T. *Diabetes* 8 261 1959
- Goodman J I, Baumel, S, Frankel, L., Marcus, L. J & Wassermann S. *The Diabetic Neuropathies*. Springfield Illinois, C. C. Thomas, 1953
- Goodof I I. *Ann intern. Med.* 2 373 1945
- Guilford J P. *Fundamental Statistics in Psychology and Education* 2nd ed. New York, Mc Graw-Hill Book Comp 1950
- Guy W B. *Penn med. J* 54 1052 1951
- Glärde A. & Kugelberg, L. *Nord. Med.* 70 1252 1963
- Ha delman M. B., Morrone T G & Ghuman, B. *Arch. intern. Med.* 110 108, 1962
- Hanser W H, Tauxe, W N, Owen C A & Lipscomb P R. *Surg. Gynec. Obstet.* 112 625 1961
- Hansen, S. *Acta phthal. (Kbh.) suppl.* 16 1959
- Hare, P J. *Brit. J. Derm.* 67 365 1955
- Hecht, A., Weissenfeld S & Goldner M G. *Metabolism* 10 712 1961
- Hollander W, Reilly P, Burrows, B A J. *clin. Invest.* 40 222 1961
- Hyman C., Rapaport, S J & Paldino R. *Amer J Physiol.* 163 722, 1950
- Ikkos D & Luft, R. *Acta endocr. (Kbh.)* 25 312 1957
- Jackson W P U. *Brit. med. J* 11 690 1952
- Jordan, W. *Arch. intern. Med.* 57 307 1936
- Joslin, E. P, Root, H F, White, P & Marble A: *The Treatment of Diabetes Mellitus*. 10th ed. Philadelphia, Lea & Febiger 1959
- Keston A. *Abstr. of Papers 129th Meeting, Amer. Chem. Soc.* 31 C, 1956
- Kety S. S: *Amer J med. Sci.* 255 352, 1948
- Kety S S. *Amer Heart J* 38 321 1949
- Kety S S: *Med. Sci. Publ. J* 128 1953
- Kummadisel, P & Wilson, C. *Amer J Path.* 12 83 1936
- Kurz, O. *Arch. Augenheilk.* 110: 284 1937
- Kurt, H., Harwitz, L. J, Ginsburg, S M. & McDowell F. *Arch. Neurol. (Chic.)* 4 31 1961
- Lax, H. & Feinberg, A. W. *Circulation* 20 1106, 1959
- Lever W F. *Histopathology of the Skin* 3rd ed. Philadelphia J. B. Lippincott Comp 1961
- Lerin, K. & Linde, S. *Svenska Lak.-udn.* 59 3016 1962
- Liebow I M., Hellerstein, H. K. & Miller M. *Amer J Med.* 18 438 1955
- Lisa, J R., Magiday M., Galloway I & Hart J F: *J Amer med. Ass.* 120 192 1942
- Locke, S, Lawrence D G & Legg, M A. *Amer J Med.* 34 775 1963
- Lundback, K. *Ugeskr. Laeg* 111 1349 1949
- Lundback, K. *Long-term Diabetes*. Copenhagen Munksgaard, 1953
- Lundback, K. *Late Development in Long-term Diabetic Vascular Disease*. In ed. Diabetes Mellitus. III Kongress der Internationalen Diabetes Federation, Düsseldorf 1958 Stuttgart, G. Thieme, 1959
- Lundback, K. *Medicinsk Årbok 1960—1961*. Stockholm, A.B. Nordiska Bokhandeln 1960
- Mackay I R. & Burnett, F M. *Autoimmune Diseases*. Springfield Illinois, C. C. Thomas, 1963
- Marbl A. *Infections Diabetes*. I Joslin, E P, Root, H. F, White P & Marble, A. ed. *The Treatment of Diabetes Mellitus*. 10th ed. Philadelphia, Lea & Febiger 1959
- Marun, M. M. *Brain* 76 594 1953
- Master A. M & Lasser R P. *Blood Pressure Elevation the Elderly*. I Brest, A. N & Moyer J H. ed. *Hypertension. Recent Advances*. Philadelphia Le & Febiger 1961



FIG 4 Microangiogram,  $\times 12.5$  from normal skin of the lower extremity atrophy specimen from non-diabetic patient (E. L. Q 32). Even the capillaries in the superficial dermis are visible Microangiogram 20 of was used.



FIG 5 Microangiogram,  $\times 12.5$  from an atrophic skin of non with surrounding clinically intact skin of the lower extremity atrophy specimen from diabetic patient (A. A. Q 36). The border of the lesion are indicated. Note the abundance of cells with foamy-like spread within the patch. The surrounding tissue is poorer in cellular content. The capillaries in the superficial dermis are barely seen and the small vessels are irregular in Microangiogram 20 of was used.

- Warren, S & LeCompte P M. The Pathology of Diabetes Mellitus 3rd ed. Philadelphia, Lea & Febiger 1952
- Weiber A. *Acta chir scand. suppl.* 237 1959
- Wexler D & Branower G. *Arch. Ophthalmol* 44 539 1950
- Wilson J L., Root, H. F & Marble, A. *New Engl. J Med.* 245 513 1951
- Winter F C.: *J Amer med. Ass.* 174 143 1960
- Wise, G N. *Trans. Amer ophthal. Soc.* 54 729 1956
- Wisham, L. H. Yalow R. S & Freund, A. J. *Amer Heart J* 41 810 1951
- Wisham, L. H. & Yalow R. S. *Amer Heart J* 43 67 1952.
- Wolman H. W & Wilder R. M.: *Arch. intern Med.* 44 576 1929
- Zacks, S I. Pegues, J J & Elliott, F A. *Metabolism* 11 381 1962



FIG 4 Microangiogram,  $\times 125$  from normal skin of the lower extremity, atrophy specimen from non-diabetic patient (E. L. Q. 22). Even the papillae in the superficial dermis are visible. Microvasculature 20  $\mu$  was ed.



FIG 5 Microangiogram,  $\times 125$  from an atrophic skin lesion with surrounding clinically intact skin of the lower extremity. Atrophy specimen from diabetic patient (K. A. O. 36). The borders of the lesion are indicated. Not the abundance of vessels within the papillae. The surrounding tissue is poorer in blood vessels. The capillaries in the superficial dermis are hardly seen and the small vessels are of irregular size. Microvasculature 20  $\mu$  was ed.



FIG 6 a

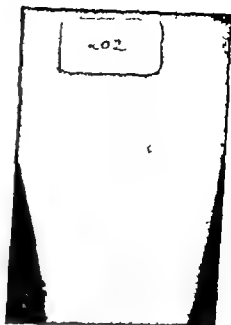


FIG 6 b



FIG 7 a



FIG 7 b

Fig 6 a J H ♂ (59) Newly discovered diabetes Primary lesion on the left leg

Fig 6 b The same lesion 10 months later now brown and atrophic

Fig 7 a K A ♂ (36) Diabetes 20 year Complications Numerous atrophic skin lesions on both legs

Fig 7 b The same patient Left leg at different angle



FIG 8



FIG 8 b.



FIG 9



FIG 10

Fig 8 B B ♂ (44) Newly discovered diabetes N complications. High incidence of diabetes in the family Brown atrophic lesions on the leg

Fig 8b The same patient Left leg at foot area

Fig 9 A S ♂ (50) Diabetes in four year V complications. Right leg, lateral and distal The atrophy clearly visible.

Fig 10 G A Q (29) Diabetes 19 year Complications Brown atrophic lesion on the left leg



FIG 6 a.



FIG 6 b



FIG 7 a.



FIG 7 b

Fig 6 a J. H. ♂ (59) Newly discovered diabetes. Primary lesions on the left leg

Fig 6 b The same lesions four months later now brown and atrophic

Fig 7 a A. A. ♂ (36) Diabetes 20 years. Complication. Numerous atrophic skin lesions on both legs

Fig 7 b The same patient. Left leg at lower angle



FIG. 8



FIG. 8b



FIG. 9



FIG. 10

Fig. 8 B. B. ♂ (44) Newly discovered diabetes & complications. High incidence of diabetes in the family. Brown atrophic lesions on the legs.

Fig. 8b The same patient. Left leg - close range.

Fig. 9 A. S. ♂ (50) Diabetes in four years. N. complication. Right leg. Lateral and distal. The atrophy is clearly visible.

Fig. 10 G. A. Q. (29). Diabetes 19 years. Complications. Brown atrophic lesions on the left leg.





FIG 11



FIG 12



FIG 13 a



FIG 13 b

Fig 11 V P ♂ (34) Newly discovered diabetes Retinopathy Left lg lateral Atrophy and pigmentation clearly seen.

Fig 12 R. A. Q (40) Diabetes 23 year Neuropathy Left lg lateral Lateral atrophy of the lesions Atrophy pronounced at some lesions

Fig 13a. P B ♂ (68) Diabetes 11 year No complications. Note the brown atrophic lesion in the middle of the picture

Fig 13b The same patient two years later The enlarged lesions are the same as seen in 13a Note the appearance of new lesions



FIG. 14 a.



FIG. 14 b.

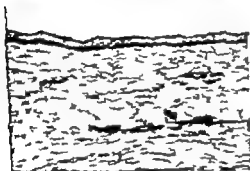


FIG. 15



FIG. 16

Fig. 14 Y G O (42)  
Diabetes 15 years.  
Retinopathy Brown  
atrophic lesions in  
linear pattern.

Fig. 14b The same  
patient 16 months later  
The lesion in 14 have  
not disappeared and new  
ones have arisen.

Fig. 15 E. M. ♂ (62)  
Diabetes 4 years. Comp-  
lications Autopsy  
specimen. Atrophic skin  
low PAS-staining X  
200. Epidermis is atrophic  
The apillaris are  
strongly PAS-positive.

Fig. 16 N. L. ♂ (60)  
Diabetes 4 years.  
Retinopathy Autopsy  
specimen. A marked  
PAS-positive apillary  
in the superficial part of  
the dermis PAS-negative.  
X 520.



FIG 11



FIG 12



FIG 13 a



FIG 13 b

Fig 11 V P ♂ (54) Newly discovered diabetes. Retinopathy Left leg lateral. Atrophy and pigmentation clearly seen

Fig 12 R A ♀ (40) Diabetes 23 year Neuropathy Left leg lateral Linear arrangement of the lesions Atrophy is pronounced in some lesions

Fig 13 P B ♂ (68) Diabetes 11 year No complications Note the brown atrophic lesions in the middle of the picture

Fig 13 b The same patient two years later The enlarged lesions are the same as seen in 13 a. Note the appearance of new lesion



FIG. IV a.



FIG. IV b.



FIG. IV c.

Fig IV a, IV b and IV c. B. W. ♂ (34) Diabetes 21 years. Complications. Fluorescence micrographs  $\times 320$  Skin from the lower leg. Punch biopsy Epidermis at the top of the pictures. a. and b. Specific fluorescence in the small vessels in the superficial part of the dermis. It corresponded to PAS-positive capillaries. In the c. from the same specimen. Fluorescence of the capillaries is entirely missing.



FIG I

Fig. I J N ♂ (50) Newly discovered diabetes. No complications. Right leg lateral. Numerous atrophic brown lesions.

Fig. II a and b Fluorescence micrographs. Atrophic skin lesion from the patient in Fig. I. Punch biopsy. Epidermis at the top of the picture. a. Specific fluorescence in the basal layer of epidermis. X 800. b. Same specimen. X 320. Inhibition of the specific fluorescence.



FIG II a



FIG II b

Fig. III L. E. Q. (50) Systemic lupus erythematosus for 18 years. Fluorescence micrograph. X 320. Skin from the lower leg. Autopsy specimen. Epidermis at the right in the picture. Note same fluorescent band in the basal layer of epidermis as in Fig. II a. Unspecific fluorescence in the horny layer of epidermis.



FIG III



FIG. IV a.



FIG. IV b



FIG. IV c.

Fig IV a, IV b and IV c. B. W. ♂ (34) Diabetes 21 year Complications. Fluorescent micrograph  $\times 332$ . Skin from the lower leg. Punch biopsy Epidermis at the top of the picture a. and b Specific fluorescence in the small spots in the superficial part of the dermis is corresponded to PAS-positive apillaries. Inhibition from the same specimen. Fluorescence of the apillaries is entirely inhibited.

S M S. MEDICAL COLLEGE

DUE DATE SLIP

This book is to be returned on or  
marked below —

A fine of annas 1000 will be charged  
if the book is kept over

--	--	--

# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 422

---

## NET CATION EQUIVALENCY (‘BASE BINDING POWER’) OF THE PLASMA PROTEINS

A STUDY OF ION-PROTEIN INTERACTION IN HUMAN PLASMA  
BY MEANS OF  
IN VIVO ULTRAFILTRATION AND EQUILIBRIUM DIALYSIS

*by*

A. M. VAN LEEUWEN

ACCOMPANIES VOL. 176



1

1

1

# ACTA MEDICA SCANDINAVICA .

SUPPLEMENTUM 422

---

## NET CATION EQUIVALENCY (‘BASE BINDING POWER’) OF THE PLASMA PROTEINS

A STUDY OF ION-PROTEIN INTERACTION IN HUMAN PLASMA  
BY MEANS OF  
IN VIVO ULTRAFILTRATION AND EQUILIBRIUM DIALYSIS

by

A. M. VAN LEEUWEN

ACCOMPANIES VOL. 176

# ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv founded in 1869 by Axel Key. The first volume of Acta Medica Scandinavica is therefore numbered LII (52).

The chief editors have been Axel Key 1869—1900, C. G. Santesson 1901—1915, I. Holmgren 1916—1957 and Birger Strandell 1958 to date.

Acta Medica Scandinavica publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board. Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the editor at the address below.

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that, if accepted, it will not be republished in any other journal in the same or similar form, without the Editor's written consent.

Papers will be published in English, French or German at the authors' choice, followed by a summary in English.

The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left hand margin.

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author. No paper shall exceed 24 printed pages.

## Subscription

The annual subscription to the journal covering two volumes, each of 6 numbers, is 140 Sw. crowns or U.S. \$ 27.25 including postage in the Scandinavian countries and in Holland 120 Sw. crowns.

*Address for subscriptions and all communications*

ACTA MEDICA SCANDINAVICA

P.O. Box 2052 Stockholm 2

---

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

**NET CATION EQUIVALENCY  
(BASE BINDING POWER)  
OF THE PLASMA PROTEINS**



**NET CATION EQUIVALENCY  
( BASE BINDING POWER )  
OF THE PLASMA PROTEINS**

**A STUDY OF ION-PROTEIN INTERACTION IN HUMAN PLASMA  
BY MEANS OF  
IN VIVO ULTRAFILTRATION AND EQUILIBRIUM DIALYSIS**

by

**A. M. VAN LEEUWEN**

**1964  
AMSTERDAM**



*To my parents*



1964  
SCHELTEMA & HOLKEMA N.V.  
AMSTERDAM

*This publication has been made possible by the financial support of  
The Netherlands Organisation for the Advancement of Pure Research (Z.W.O.)*

# Contents

GENERAL INTRODUCTION	ix
LIST OF ABBREVIATIONS	xii

## PART I

CHAPTER I - <i>Physico-chemical introduction to the study of the interaction between inorganic ions and proteins in plasma, with a definition of terms</i>	3
Water ions and proteins	3
Discussion of the interaction between small ions and proteins and definition of the terms used to describe this interaction	9
The effect of protein on the distribution of filtrable ions across a semipermeable membrane and the use of this effect in the study of protein-bound ion	17
Review of data concerning the interaction between albumin and inorganic ions	25
The measurement in plasma (or serum) of protein-bound ion, net protein-bound cation (NCE) and of protein net charge	30
Summary of chapter I	34

CHAPTER II - <i>Review of literature concerning the net cation equivalency of plasma proteins the normal concentration of the main filtrable plasma ions and the distribution of the latter between plasma and interstitial fluid</i>	36
NCE of plasma proteins (normal plasma)	36
NCE of plasma proteins (pathological plasma)	48
Distribution of the main filtrable ions between normal plasma and interstitial fluid, and conclusions concerning the interaction of these ions with plasma proteins	50
Interaction of filtrable ions with plasma proteins under pathological conditions	56
Summary of chapter II	57

## PART II

CHAPTER III - <i>Chemical analysis</i>	61
Syringes, glassware and stoppers	61
Obtaining and handling of samples	61
Chemical methods	63
Water content [63] Protein (macro-Kjeldahl) [64] Protein (biuret method) [65] Protein fractions [66] Na and K [69] Ca and Mg [76] [77] Total CO [80] pH [82] $\text{HCO}_3^-$ [83] $\text{P}_{\text{co}_2}$ [87] Inorganic phosphate [87] Lactate [87] Haemoglobin [87] $\text{HbO}_2$ $\angle$ [87]	
Summary of chapter III	89



## General Introduction

In the course of an earlier investigation in which we assisted GERBRANDY we became increasingly intrigued by the so-called "base binding power" of the plasma proteins.

In the literature on ion-protein interaction the term 'binding' is not always well defined. It is confusing to read first one author who concludes that Na is bound by plasma proteins, then another who concludes that it is not, and finally find that the authors are in perfect agreement but use the term binding in a different sense.

Apart from its ambiguity as a term, the "base binding power" of the plasma proteins is characterised by the scarcity of facts concerning its magnitude. The normal value always quoted - 16 mEq/L plasma - is based on a single publication by VAN SLYKE *et al* in 1928. Although in itself an impressive example of detailed and exact experimental evidence, its usefulness must be limited by the fact that the substrates studied were albumin and globulin obtained by ammoniumsulfate fractionation of two horse sera and one batch of pooled human serum. In the light of present knowledge concerning protein chemistry it is questionable whether the results thus obtained are valid for plasma.

This has a bearing on the data concerning the distribution of ions across a semi-permeable membrane. The "base binding power" of the plasma proteins is one of the main factors determining the ion distribution across the capillary wall and the value found by Van Slyke *et al* has been used to calculate the Donnan ratio. But whether the ratio thus obtained is correct is difficult to assess because the inorganic ions have different concentration ratios, and it is unknown which one - if any - of these ratios is identical with the Donnan ratio. The differences in concentration ratio are presumably caused by differences in the nature of the ion-protein bond. Consequently comparison of the concentration ratios observed in dialysis experiments with the Donnan ratio expected provides information concerning the interaction between ion and protein as NORTHRUP & KUMTZ could demonstrate for gelatin (1925). A similar approach is possible with the plasma proteins and has indeed been used by GREENE & POWER (1931) but their conclusions are open to criticism because the value of the Donnan ratio for the equilibrium plasma-interstitial fluid was not known then (and is not known now) with sufficient accuracy. This leads back to the questions concerning the nature and the magnitude of the "base binding power" of the plasma proteins.

If the data are already scarce and inadequate for normal plasma, they are even more so for plasma in disease. But the careful observations of GUTHMAN *et al* (1936) appear to justify their negative conclusion concerning the validity of the values given by Van Slyke *et al* in the case of plasma with an abnormal protein composition.

CHAPTER IV – <i>In vivo</i> ultrafiltration	90
Technique and calculations	91
A study of the subjective and objective effects of prolonged compression of the upper arm	93
Final evaluation of the assumptions made with regard to the data obtained by means of <i>in vivo</i> ultrafiltration	114
Summary of chapter IV	117
CHAPTER V – <i>Equilibrium dialysis</i>	119
Technique and apparatus	119
Sampling, and treatment of analytical data	124
Sources of errors in technique and observations	125
Summary of chapter V	138

### PART III

CHAPTER VI – <i>Results obtained with in vivo ultrafiltration (including normal values for the plasma ions)</i>	143
Normal subjects	143
Patients	154

CHAPTER VII – <i>Results obtained with equilibrium dialysis</i>	160
Serum from normal subjects	160
NCE and $\Delta$ cation [165] – Concentration ratios [169]	
Serum from patients	171
Purified protein fractions	173
NCE and $\Delta$ cation [176] – Concentration ratios [179]	

CHAPTER VIII – <i>Comparison and discussion of the results obtained by means of in vivo ultrafiltration and equilibrium dialysis</i>	180
Net cation equivalency (NCE)	180
Normal subjects [180] – Patients and purified protein fractions [181]	
Quantity of protein-bound cation	182
Normal subjects [182] – Patients and purified protein fractions [184]	
The Donnan ratio and the nature of ion-protein binding – Negative net charge of protein	185

CHAPTER IX – <i>Summing up</i>	191
LIST OF BIBLIOGRAPHICAL REFERENCES	203

ADDENDUM	
by Dr C. G. G. VAN HERK	207

## General Introduction

In the course of an earlier investigation in which we assisted GERBRANDY we became increasingly intrigued by the so-called 'base binding power' of the plasma proteins.

In the literature on ion-protein interaction the term 'binding' is not always well defined. It is confusing to read first one author who concludes that Na is bound by plasma proteins, then another who concludes that it is not, and finally find that the authors are in perfect agreement but use the term binding in a different sense.

Apart from its ambiguity as a term, the 'base binding power' of the plasma proteins is characterised by the scarcity of facts concerning its magnitude. The normal value always quoted - 16 mEq/L plasma - is based on a single publication by VAN SLYKE *et al* in 1928. Although in itself an impressive example of detailed and exact experimental evidence, its usefulness must be limited by the fact that the substrates studied were albumin and globulin obtained by ammoniumsulfate fractionation of two horse sera and one batch of pooled human serum. In the light of present knowledge concerning protein chemistry it is questionable whether the results thus obtained are valid for plasma.

This has a bearing on the data concerning the distribution of ions across a semi-permeable membrane. The 'base binding power' of the plasma proteins is one of the main factors determining the ion distribution across the capillary wall and the value found by Van Slyke *et al* has been used to calculate the Donnan ratio. But whether the ratio thus obtained is correct is difficult to assess because the inorganic ions have different concentration ratios, and it is unknown which one - if any - of these ratios is identical with the Donnan ratio. The differences in concentration ratio are presumably caused by differences in the nature of the ion-protein bond. Consequently comparison of the concentration ratios observed in dialysis experiments with the Donnan ratio expected provides information concerning the interaction between ion and protein as NORTHROP & KUNITZ could demonstrate for gelatin (1925). A similar approach is possible with the plasma proteins and has indeed been used by GREENE & POWER (1911) but their conclusions are open to criticism because the value of the Donnan ratio for the equilibrium plasma-interstitial fluid was not known then (and is not known now) with sufficient accuracy. This leads back to the questions concerning the nature and the magnitude of the 'base binding power' of the plasma proteins.

If the data are already scarce and inadequate for normal plasma, they are even more so for plasma in disease. But the careful observations of GUTMAN *et al* (1936) appear to justify their negative conclusion concerning the validity of the values given by Van Slyke *et al* in the case of plasma with an abnormal protein composition.

The present study is the outcome of an attempt to understand the nature of the base binding power of the plasma proteins and to determine its magnitude both in health and in various states of disease under conditions which imitate the situation *in vivo* as closely as possible. This was done by studying the effect of changes in the protein concentration of plasma on the concentrations of Na, K, Ca, Mg, Cl and  $\text{HCO}_3$ . For this purpose we made use of two techniques.

*In vivo ultrafiltration* the principle of which we learned from GERBRANDY. The protein concentration of venous plasma is caused to increase by producing venous congestion in the forearm. By correlating the change in protein concentration with the concomitant changes in the concentration of the ions mentioned above, information can be obtained concerning the interaction between these ions and the plasma proteins. The accuracy required for our purpose made it necessary to study the effect of congestion on the composition of other components of venous blood as well.

*Equilibrium dialysis* in which fresh serum or purified proteins fractions were dialysed *in vitro* at 38°C through a cellulose membrane against synthetic interstitial fluid. The difference in ion concentration inside and outside the membrane can be correlated with the protein concentration inside, and the concentration ratio can be calculated for each ion. Also the effect of change in pH can be studied.

Since the base binding power of the plasma proteins was obtained by summing the concentrations of the individual cations and subtracting the sum concentration of chloride and bicarbonate, the errors of at least 7 individual determinations cumulate. Much of our effort had to be devoted to keeping these errors as small as possible; this is reflected in the space taken up by the description and discussion of the methods of chemical analysis and of the experimental techniques.

The subject matter will be presented in three parts.

Part I is concerned with definitions and with the physico-chemical background of the methods used (chapter I), with data from the literature regarding the base binding power of plasma proteins (for which the term net cation equivalency shall be used), and the concentration ratio of the various small ions across the capillary wall (chapter II).

In Part II we shall describe and discuss methods of chemical analysis (chapter III), the technique of *in vivo* ultrafiltration (chapter IV) and that of equilibrium dialysis (chapter V).

In Part III we will present the results obtained with *in vivo* ultrafiltration (chapter VI) and those obtained with equilibrium dialysis (chapter VII). In chapter VIII the results obtained with both methods are compared and conclusions are drawn with regard to the net cation equivalency of the plasma proteins, the Donnan ratio for the equilibrium plasma - interstitial fluid, the protein-bound quantities of the various inorganic ions and the nature of their interaction with protein. Chapter IX contains the summing up.

All tables with the exception of the last two (chapter IX), have been printed together in a separate booklet, which is added as an appendix.

Some further remarks must be made.

Chapter I was written in an attempt to provide the medical reader with sufficient physico-chemical data to understand the limitations of the methods used for studying ion-protein interaction. At the same time chapter I should provide the theoretical base

for the experiments and the conclusions to be presented. As a result, the first chapter has become rather extensive, even though far from complete as a physico-chemical introduction. But it will perhaps explain why we find ion-protein binding a fascinating subject.

In this study the attention will be mainly directed to the effect of plasma proteins on the inorganic plasma ions in general, and on the univalent cations and anions in particular. The bivalent cations, notably Ca, have always held most of the attention and the studies concerning the interaction between protein and Ca, recently also Mg, have been reviewed admirably and repeatedly in recent years.

The inorganic ions will be indicated by their atomic symbol. But whereas in the theoretical considerations in chapter I they are provided with the appropriate valency sign, no valency sign will be used in the later chapters where we are concerned with the ions as determined by chemical analysis.

A number of other symbols and abbreviations have been used. These are explained in chapter I and listed on the next page.

On account of our insufficient knowledge of statistical mathematics we have limited our statistical treatment to the calculation of regression lines (method of least squares) and the calculation of standard deviations for a single determination from duplicate determinations (using the equation

$$s^2 = \sum_1 (x_1 - x_2)^2 / 2n,$$

where  $n$  is the number of duplicates). However Dr. C. G. G. VAN HERK (Mathematisch Centrum in Amsterdam) was kind enough to analyse statistically the primary information obtained in the *in vivo* ultrafiltration experiments with normal subjects. He has described the results of this analysis separately (see Addendum).

Finally I am most grateful to miss J. J. VAN DAATSELAAER, Mrs. P. C. KAPTEYN-JANSSE and Mrs. C. M. DE SENERPONT DOMES-THOMASSE for their painstaking chemical analyses. Without their dedication this study would not have been possible. I am also indebted to our biochemist L. A. DE VRIES for always being ready to help and - most important - to listen.



The present study is the outcome of an attempt to understand the nature of the base binding power of the plasma proteins and to determine its magnitude both in health and in various states of disease under conditions which imitate the situation *in vivo* as closely as possible. This was done by studying the effect of changes in the protein concentration of plasma on the concentrations of Na, K, Ca, Mg, Cl and  $\text{HCO}_3$ . For this purpose we made use of two techniques.

*In vivo ultrafiltration*, the principle of which we learned from GERAUNDY. The protein concentration of venous plasma is caused to increase by producing venous congestion in the forearm. By correlating the change in protein concentration with the concomitant changes in the concentration of the ions mentioned above, information can be obtained concerning the interaction between these ions and the plasma proteins. The accuracy required for our purpose made it necessary to study the effect of congestion on the composition of other components of venous blood as well.

*Equilibrium dialysis* in which fresh serum or purified proteins fractions were dialysed *in vitro* at 38°C through a cellulose membrane against synthetic interstitial fluid. The difference in ion concentration inside and outside the membrane can be correlated with the protein concentration inside and the concentration ratio can be calculated for each ion. Also the effect of change in pH can be studied.

Since the base binding power of the plasma proteins was obtained by summing the concentrations of the individual cations and subtracting the sum concentration of chloride and bicarbonate, the errors of at least 7 individual determinations cumulate. Much of our effort had to be devoted to keeping these errors as small as possible; this is reflected in the space taken up by the description and discussion of the methods of chemical analysis and of the experimental techniques.

The subject matter will be presented in three parts.

Part I is concerned with definitions and with the physico-chemical background of the methods used (chapter I), with data from the literature regarding the base binding power of plasma proteins (for which the term net cation equivalency shall be used), and the concentration ratio of the various small ions across the capillary wall (chapter II).

In Part II we shall describe and discuss methods of chemical analysis (chapter III) the technique of *in vivo* ultrafiltration (chapter IV) and that of equilibrium dialysis (chapter V).

In Part III we will present the results obtained with *in vivo* ultrafiltration (chapter VI) and those obtained with equilibrium dialysis (chapter VII). In chapter VIII the results obtained with both methods are compared and conclusions are drawn with regard to the net cation equivalency of the plasma proteins, the Donnan ratio for the equilibrium plasma - interstitial fluid, the protein bound quantities of the various inorganic ions and the nature of their interaction with protein. Chapter IX contains the summing up.

All tables, with the exception of the last two (chapter IX), have been printed together in a separate booklet which is added as an appendix.

Some further remarks must be made.

Chapter I was written in an attempt to provide the medical reader with sufficient physico-chemical data to understand the limitations of the methods used for studying ion protein interaction. At the same time chapter I should provide the theoretical base

## PART I

## *List of Abbreviations*

Cat, An	= cation, anion
[ ]	= concentration expressed per Kg H <sub>2</sub> O
<i>f</i>	= activity coefficient
NCE	= net cation equivalency of proteins (base binding power)
Pr <sup>-</sup>	= negative net charge of proteins
RA	= rest anions, <i>i.e.</i> anions other than chloride, bicarbonate and protein
CatPr AnPr	= cation respectively anion protein-bound in a complex type manner
cat min Cl + HCO <sub>3</sub>	= sum concentration of Na, K, Ca and Mg minus sum concentration of Cl and HCO <sub>3</sub>

The following abbreviations and symbols refer to membrane equilibria

suffix i	= inside <i>i.e.</i> protein-containing solution
suffix o	= outside <i>i.e.</i> protein-free solution
<i>r</i>	= Donnan ratio
$R_{cat} = \frac{[Cat]_i}{[Cat]_o}$	= concentration ratio of cation
$R_{an} = \frac{[An]_i}{[An]_o}$	= concentration ratio of anion
$\Delta Cat$	= $[Cat]_i - [Cat]_o$

## PART I



## CHAPTER I

### *Physico-chemical introduction to the study of the interaction between inorganic ions and proteins in plasma with a definition of terms*

When studying the interaction between proteins and small ions in solutions such as plasma assumptions of a simplifying nature must be made. To begin with we will have to consider plasma as being essentially an aqueous solution containing on the one hand the inorganic ions  $H^+$ ,  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Cl^-$  and  $HCO_3^-$  and on the other proteins. But other assumptions will have to be made as well and when the nature and limitations of these assumptions are not recognised the interpretation of the experimental data may be seriously in error as the literature reveals. One example is provided by those studies concerning membrane equilibrium in which no distinction is made between the Donnan ratio on the one hand and the concentration ratio of a particular ion on the other. Another example is found in the fact that the base binding power of the plasma proteins is quite generally accepted as being identical with the negative net charge of the proteins. This is not so as we hope to show.

This chapter is the outcome of an attempt to realise the assumptions underlying the present study and the extent to which they affect the interpretation of the experimental data. We will first consider the characteristics of the plasma constituents with which we are concerned (water ions and proteins), and the interactions between the latter two. Ion-protein interaction will then be defined in a manner suited to the purpose of our study and those aspects of membrane equilibria will be discussed which are necessary for the interpretation of our experiments. We will then review the literature concerning ion binding by albumin, this being the plasma protein most studied. Finally we will consider what information concerning ion-protein binding we may expect to obtain from our experiments with plasma.

Since a review of general principles is always based on concepts and facts gathered over a long period by a multitude of scientists, we shall refrain from quoting authors in particular unless a definite investigation is at the back of an example or statement. But we should like to mention here BOON (1956) and EDZELL & WYMAN (1958), whose introductions to physical biochemistry have been most helpful.

## WATER, IONS AND PROTEINS

### WATER

In aqueous solutions the water molecules largely determine the characteristics of the solution. Because of their structure ( $O \begin{smallmatrix} \diagup H \\ \diagdown H \end{smallmatrix}$ ) the molecules act as dipoles and tend to

hang together. *Solutes carrying an electrostatic charge (ions) attract the water molecules. These arrange themselves around the sites of charge (hydration) and in doing so diminish the electrostatic forces acting between the charged particles in solution.  $H^+$  ions (protons) and  $OH^-$  ions occupy a special position owing to the fact that water molecules are not completely stable. The negative pole of a  $H_2O$  molecule (the side where the O-atom is situated) tends to attract  $H^+$  ions from the positive poles of neighbouring  $H_2O$  molecules (the side where the H atoms are located) In reality therefore water must be described by the equilibrium equation*



*In pure water the amount of ( $H_3OH^+$ ) and ( $OH^-$ ) is extremely small. If  $H^+$  ions are added they will attach themselves to  $H_2O$  molecules and move through the solution from one molecule to another. This explains the high mobility of  $H^+$  ions and their important role in aqueous solutions.*

### Concerning concentrations

*Water being the solvent the amount of solutes must be expressed per litre or per Kg  $H_2O$  preferably the latter because then the solvent can be considered in terms of molecules (1000 g  $H_2O$  = 55.55 moles  $H_2O$ ). Concentrations per Kg  $H_2O$  are indicated by placing the molecular symbol of the solute between brackets [ ]. When molecular interactions are studied the concentration of the solutes is expressed in (milli)moles per Kg  $H_2O$  when ion-ion interactions are investigated the concentration is expressed in (milli) equivalents per Kg  $H_2O$ .*

*The concentration of the  $H^+$  ions is not expressed as such but in pH values. For practical purposes one can consider  $pH = -\log [H^+]$*

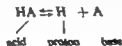
### IONS

*An ion is a substance which carries an electrostatic charge. Classical examples are the inorganic ions. These are atoms which have acquired a positive or negative charge by losing or taking up electrons. Positively charged ions tend to form stable crystals with negatively charged ions (for example  $NaCl$ ,  $KHCO_3$ ,  $CaCl_2$ ). But when dissolved in water these substances dissociate in their component ions because of the hydration and the resulting insulation of the ions. The relative independence thus obtained by the individual ions ( $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ ,  $Cl^-$ ) is manifest from the fact that when an electrostatic field is applied to the solution the component ions of the original substance move in opposite direction and may leave the solution at the electrodes. Hence the name electrolytes and the denomination of positively charged ions by the term cation and of negatively charged ions by the term anion. Considered individually ions have a remarkable freedom of movement but as a group they are subject to the restriction that the total positive (cationic) charge must equal the total negative (anionic) charge in the solution (law of electroneutrality).*

*The inorganic ions may differ considerably in their behaviour towards other substances as a result of differences in sign and magnitude (valency) of their electrostatic charge and in the size of the ion. An example is the tendency to attract water molecules, which is strongest when the ion radius is small and the valency high.*

### Ionogenic group - Acid - Base

The term ion or ionic substance has been extended to groups of atoms which have acquired a positive or negative electrostatic charge by a process of association or dissociation. An arrangement of atoms which is capable of doing so is indicated by the term ionogenic group. The essential point about ionogenic groups is that they may or may not carry an electrostatic charge, according to the circumstances. Examples are provided by the acids and bases. BRØNSTED defines an acid as a substance which becomes a base by losing an  $H^+$ -ion (protondonator), a base as a substance which becomes an acid by taking up an  $H^+$ -ion. The  $H^+$ -ion has a central position in the definition and the relation between acid and base can best be described by the equation



In this example the acid is the ionogenic substance. The ease with which an acid will dissociate into  $H^+$  and  $A^-$  is indicated by the value of the *dissociation constant* which is defined by the equation

$$\frac{[H^+][A^-]}{[HA]} = K_a$$

The higher the value of  $K_a$  the stronger the acid  $HA$  (and the weaker the base  $A^-$ ). Solutions containing approximately equal amounts of a weak acid and its base (or a weak base and its acid) tend to keep the  $H^+$ -ion concentration constant (buffer solutions). To fit the pH notation the equilibrium is in the case of buffers generally described as

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \quad (\text{where } pK_a = -\log K_a)$$

The dissociation constant is not really a constant. It depends on the temperature of the solution, on the characteristics of the other solutes, on the density of acid molecules (their molal concentration). When a molecule contains more than one ionogenic group the tendency to dissociate is strongly influenced by the spatial arrangement of the groups.

#### Spatial arrangement of ionogenic groups

Its influence may be illustrated by comparing phosphoric acid and citric acid. Both carry 3 acid groups and can therefore donate 3  $H^+$ -ions. But in the case of phosphoric acid all the ionogenic  $-OH$  groups are attached to the same P atom, whereas in the case of citric acid each of the ionogenic  $-COOH$  groups is connected to a different C atom, which are themselves separated by another C atom. The dissociation of the first group and the concomitant acquisition of a negative charge will in the case of phosphoric acid strongly depress the tendency to dissociation for the remaining groups. But in the case of citric acid the distance is sufficiently large to diminish the influence of one group on the others. The point is illustrated by comparing for both substances the constants of each of the three steps of dissociation.



	$K_1$	$K_2$	$K_3$
Phosphoric acid (at 18°)	$1.1 \times 10^{-3}$	$7.5 \times 10^{-8}$	$4.8 \times 10^{-13}$
Citric acid (at 25°)	$8.4 \times 10^{-4}$	$1.8 \times 10^{-5}$	$4 \times 10^{-6}$

(Taken from *Handbook of Chemistry and Physics* 30th ed.)

This example stresses the fact that in the case of large molecules carrying several univalent dissociated ionogenic groups the molecule should be regarded as being essentially a number of univalent ions instead of one single polyvalent ion

**Ionic substances which can simultaneously carry a positive and a negative charge (amphoteric substances) – Net charge**

The classic example is provided by the amino acids. Amino acids are essentially a combination of a  $-\text{COOH}$  group, a  $-\text{NH}_2$  group and an alkyl group which may or may not carry other ionogenic groups. The  $-\text{NH}_2$  group is in the  $\alpha$  position i.e. it is connected to the same C atom as the  $-\text{COOH}$  group and whereas the carboxyl group is a rather strong acid the  $-\alpha\text{NH}_2$  group is a weak base. If we consider the simplest amino acid, glycine, we find that at very low pH values the carboxyl group is not dissociated and therefore carries no charge, but that the amino group is associated with a  $\text{H}^+$  ion and thus carries a positive charge ( $-\text{H}_2\text{NH}^+$ ) (fig. 1). When we add NaOH

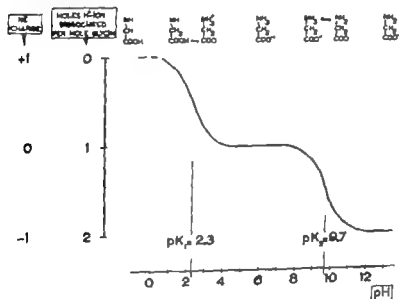


Fig. 1 Schematic representation of titration curve of glycine.

till the pH is increased to a value higher than the pK of the  $-\text{COOH}$  group but still below the pK of the  $-\text{H}_2\text{NH}^+$  group the amino acid ion still carries a positive charge, but in addition now also a negative one. If finally the pH increases to values well above the value of the pK of the  $-\text{H}_2\text{NH}^+$  group the latter loses its  $\text{H}^+$ -ion and the amino acid ion carries only a negative charge. Thus while first one and then the second  $\text{H}^+$  ion is dissociated the charge on the ion changes from one unit of positive charge to a combination of one unit of positive and one unit of negative charge, and finally to one unit of negative charge. The arithmetic sum of positive and negative charges on the ion – its net charge – therefore changes from  $+1 \rightarrow 0 \rightarrow -1$ . Fig. 1 is a (schematic) repre-

presentation of such a titration curve. At the ordinate are indicated both the number of H<sup>+</sup> ions dissociated per molecule and the net charge of the molecule. The pH trajectory over which the net charge remains zero is the *iso-electric pH* trajectory. In this - simple - case it is a pH range, but if the number and variety of ionogenic groups on a molecule increase the pH range over which the amphoretic ion has a net charge of zero narrows down until for proteins it has become one fairly circumscribed pH value, the *iso-electric pH* (IEP). At this point the effect of an amphoteric ion on the balance between the total cationic and anionic charges in the solution is the same as that of a neutral molecule.

A number of amino acids carries other ionogenic groups beside the primary —COOH and the —NH<sub>2</sub> group. Because of the difference in dissociation constants of these groups the titration curves of polypeptides take on a rather complicated form and the number of H<sup>+</sup> ions which can successively be dissociated with increasing pH can become very large.

#### The size of ionic substances - Filtrable and non-filtrable ions - Free filtrable ion

Ionic size acquires special importance when an ionic solution is surrounded by a porous membrane. If the pores are sufficiently narrow the small ions can pass through the membrane but the macromolecular ions can not. We will therefore designate ions as *filtrable* or *non-filtrable* according to whether their size does or does not permit them to pass through the pores of such a semipermeable membrane (collodion, capillary membrane).

Although filtrable ions such as Na<sup>+</sup> Ca<sup>2+</sup> etc. are refrained from passing through semipermeable membrane when they have become attached to non-filtrable substances. The term 'filtrable' refers therefore to the potential rather than to the actual behaviour of an ion. A filtrable ion not bound by non-filtrable substance will be indicated as 'free'.

### PROTEINS

Proteins are highly intricate, amphoteric macromolecules. They are ionic substances, but size and structure put them in a class apart.

Because of their size the proteins are perfect examples of non-filtrable ions. They can therefore be separated from the filtrable ions present in the same solution by means of dialysis or filtration through a suitable semipermeable membrane. However the presence of protein on only one side of the membrane causes the filtrable ions to distribute themselves in an unequal fashion between the two solutions divided by the membrane. The resulting Donnan equilibrium is of considerable interest and will be discussed in the third part of this chapter.

The structure of the proteins allows for an enormous diversity in number, nature and spatial arrangement of ionogenic groups. The backbone of the protein molecules is a chain of peptide bonds which are formed by the reaction of the primary —COOH group of one amino acid with the —NH<sub>2</sub> group of another. From this backbone structure the remaining parts of the amino acid molecules project as side chains. Since the bond between the nitrogen atom and the carbon atom carrying the side chain can rotate in regard to the plane in which the other atoms of the peptide bond are situated, the chain of peptide bonds can assume different shapes. The two main variations are the linear arrangement found in proteins like keratin and fibrin, and the spiral or helix arrangement

which is typical for the globular proteins to which all other plasma proteins belong. Especially with the latter type of proteins intricate three-dimensional structures of great molecular weight can be formed by alternating clockwise and counterclockwise rotating helices with parts that show a linear arrangement.

Among the factors responsible for the shape of a protein molecule are the nature and position of the side chains. Those carrying ionogenic groups tend to project into the surrounding water whereas the purely aliphatic side chains strive to get away from the water and huddle together in the centre of the protein molecule, probably creating a hydrophobic core in some of the proteins.

The ionogenic groups most frequently encountered are the carboxyl groups and the amino groups, but imidazole, phenolic, guanidin and sulphhydryl groups are present as well. The values of the dissociation constants of these groups range from about  $10^{-3}$  to  $10^{-12}$  which makes the titration curves of most proteins rather complicated. Of special interest is the spacing of the ionogenic groups on the protein surface. When the carboxyl groups for instance are wide apart they represent at a pH value of 7.4 truly univalent sites of charge. But when there is a high density of carboxyl groups in a certain area of the protein surface this area will at pH 7.4 show a considerable accumulation of negative electrostatic charge and the effect on the surrounding solution will be comparable to that of bi- or even trivalent sites of charge. Although in the case of the plasma proteins practically nothing is known about spatial arrangements of ionogenic groups these different possibilities must be kept in mind when considering ion protein interaction.

Quite a different force, but one which contributes greatly to the shape of the protein molecules, results from the strong dipole nature of the peptide bond, where the  $>NH$  group acts as positive and the  $>C=O$  group as negative pole. This favours the attraction of the  $-NH$  group in one peptide bond by the  $>C=O$  group in another peptide bond (located for instance in a parallel coil of the helix structure) by making them share the H-atom of the amino group. These hydrogen bonds are very unstable and easily influenced by factors like temperature, concentration of inorganic ions in the solution, the pH etc.

All this explains why most of the plasma proteins are very sensitive to relatively small alterations in the composition and temperature of the solution. Furthermore both the number of and variety in plasma proteins are remarkably large. They have molecular weights ranging from about 40 000 to about 1 000 000 their isoelectric points range from 2.7 to 7.3 they have a carbohydrate content varying from insignificant to something like 40 % and a lipid content varying from 1 to 99 % of the molecular weight. *conulo-plasmin* has a highly specific affinity for  $Cu^{2+}$  transferrin for  $Fe^{3+}$ . The amount of knowledge which has been gathered concerning the plasma proteins is impressive. Yet the number of plasma proteins which are now well defined as to composition, structure and physico-chemical characteristics is still small notwithstanding highly ingenious research in which the most varied chemical and physical methods are used. This is due to the difficulty of obtaining a pure protein without altering its properties to such an extent that it becomes a mere caricature of itself. The fact that albumin is the one plasma protein about which most is known is partly due to its robustness. But the example of albumin may also serve to show that *in vitro* experiments with purified proteins are not necessarily representative for the behaviour of the protein as present in plasma. FOSTER (1960) in discussing the purity of albumin preparations remarks upon the fact that even in the best crystallised preparations the albumin was found to contain one or a few

moles of fatty acid contamination per mole of protein and that the complete removal of these by special techniques results in a protein preparation which crystallizes with difficulty. Apparently the contamination with fatty acids modifies the physical behaviour of the plasma albumin and Foster concludes by suggesting that certain differences observed in behaviour between bovine and human albumin may reside in differences in the level of fatty acids contamination. Contamination is the operative word here. It suggests a nuisance - which, of course, it is to physical chemists who are in the first place interested in defining the characteristics of pure substances. But the biologist might with equal right consider the removal of the last molecules of 'contaminating' fatty acids the final step in the process of divesting the plasma albumin from its biological characteristics.

Clearly caution is needed when using results obtained in experiments with purified proteins to explain phenomena observed in the study of plasma. With this restriction the results obtained with albumin preparations are of considerable interest and will be reviewed in the fourth part of this chapter.

## DISCUSSION OF THE INTERACTION BETWEEN SMALL IONS AND PROTEINS AND DEFINITION OF THE TERMS USED TO DESCRIBE THIS INTERACTION

### INTERACTION BETWEEN SMALL IONS (SALT TYPE OF INTERACTION)

#### Dissociation

In water the component ions of an electrolyte tend to dissociate *i.e.* to be separated by interposition of water molecules. For strong electrolytes (such as NaCl) this dissociation is considered to be complete, *i.e.* all ions are separated by water molecules. However the ions still influence each other through electrostatic forces and as a result a solution of strong electrolytes behaves differently from what the theory of complete dissociation leads one to expect.

#### Activity - Activity coefficient - Ionic strength

All theoretical considerations concerning the properties of a solution are based on the behaviour of an ideal gas. The molecules of such a hypothetical gas are *inert* to each other and consequently have complete freedom of movement as long as they do not collide. An ideal gas obeys strictly the dictates of the general gas equation. By assuming the solutes in a solution to behave as the molecules of an ideal gas, characteristics of the solution such as osmotic force can be considered in terms of the gas equation. However the more concentrated the solutions studied, the more the experimental values for freezing point etc. tend to deviate from the values calculated by inserting the molar concentrations of the solute in the equations derived from gas kinetics. Since freezing point, vapour pressure, conductivity (in the case of electrolytes) depend on the number of 'free' solute particles per unit volume, the observed deviation suggests that there are less 'free' particles than one would expect from the molar concentration and the theory of complete dissociation of salts. This phenomenon can be explained as follows. No solute is completely inert, least of all the ions in an electrolyte solution. The insulating effect of the water molecules diminishes the electrostatic attraction to the extent that

the ions no longer adhere spatially but some electrostatic attraction between the ions will remain until their concentration becomes infinitely low. Conversely the higher their concentration the more the ions will interact the solution will behave less and less like an ideal gas because for each ion the freedom of movement is increasingly restricted. The effect is the same as when there would be a decrease in the number of completely independent particles per unit volume.

One can therefore reconcile the theory of molecular kinetics with the experimental data by considering the hypothetical quantity of completely independent solute particles per unit volume instead of the molar concentrations obtained by chemical analysis. This hypothetical quantity which makes the experimental data fit in with the theory of ideal gas behaviour has been termed by LEWIS the *activity* of a solute. Its relation to the concentration is given by the *activity coefficient*  $f$  where  $f = \text{activity}/\text{concentration}$ . By definition for all solutions,  $f = 1.0$  at infinite dilution. From the above considerations it must be concluded that for an electrolyte solution the activity coefficient is related to – and thus a measure for – the interaction between the ions. The stronger the interaction the lower the activity coefficient of the solution. The magnitude of the latter depends therefore on the concentration of the ions, on their valencies, on the temperature of the solution to name the most important factors.

The large effect of valency is demonstrated by the following data from LEWIS and RANDALL (1923). At 25°C and a molal concentration of 0.1 the value of  $f$  is for NaCl 0.798, for BaCl<sub>2</sub> 0.501 for MgSO<sub>4</sub> 0.166(1).

A measure for the concentrations and valencies of all ions present in a solution is found in the *ionic strength* of that solution another useful concept of Lewis.

The ionic strength ( $\mu$ ) is defined by the equation

$$\mu = \frac{1}{2} \sum C_a Z_a^2 + \frac{1}{2} \sum C_b Z_b^2$$

where  $C$  indicates molar concentrations,  $Z$  valency and the suffix  $a, b, \dots$  the various types of ion present in the solution. For plasma the ionic strength is generally calculated as 0.16 on the assumption that in regard to ionic strength the proteins act as univalent ions the number of which is equal to the numerical value of protein net charge.

### Determination of activity coefficient

For solutions containing one or two types of electrolytes the value of  $f$  has been determined experimentally from measurements of freezing point, vapour pressure etc. It is also possible to estimate  $f$  with the aid of equations derived by DEBYE and HÜCKEL. In these equations figure such factors as ionic strength, ion valency, ion diameter, dielectric constant of solvent etc. For dilute solutions (> 0.1 M/L) containing not too many different ions  $f$  can be estimated from ionic strength and valencies by means of a relatively simple equation. At higher concentrations and for polyvalent ions the Debye-Hückel equations become very complicated and the values calculated less accurate.

Until now we have discussed the activity coefficient of a solution. For a single type of ion  $f$  can be estimated in two ways. The first one rests on the assumption proposed by MACINNES that the activities of K<sup>+</sup> and of Cl<sup>-</sup> ions are equal and have the same value as the activity found for a solution of KCl. One can then calculate the activity coefficient for other types of ion by a process of comparison when it is further assumed that in a dilute solution containing various electrolytes the activity coefficient of any ion depends

*solubility on the ionic strength of the solution*. The latter assumption was made by Lewis, who stressed its hypothetical nature and made the restriction that a solution can no longer be considered dilute when its ionic strength increases beyond values of a few hundredths to a few tenths, according to the valency of the ions. It should be noted that Lewis studied solutions containing no macromolecules.

The second way of measuring the activity coefficient of a single type of ion is provided by the principle of the ion-specific electrodes. Essentially these contain the parent atoms of the particular ion for which they are sensitive. When placed in a solution containing this ion an equilibrium will be established between the rates at which ions turn into atoms and vice versa. A change in the activity of the ions in the solution will therefore cause a corresponding change in the rate at which electrons are released from (or taken up by) the electrode. This phenomenon can be measured by incorporating solution and ion-specific electrode in a measuring circuit which contains a potentiometer, a reference electrode in a reference solution, and a liquid junction between the two solutions. If the potentiometer is to indicate correctly the changes in potential at the measuring electrode, then the potentials at the reference electrode and at the junction must have a constant value regardless of the solutions studied. This condition is not easily fulfilled. There are more difficulties.

The need to define  $\gamma_{\pm}$  scale with the aid of standard solutions indicates that no absolute values for ion activity can be obtained from electrometric measurements. Even the interpretation of changes in potential in terms of changes in ion activity is fraught with theoretical difficulties. Accordingly the number of assumptions necessary is already large in the case of simple salt solutions. When electrolyte solutions containing protein are studied, the uncertainties become still larger if only because apparently nothing definite is known about the effects which the presence of proteins as such has on the junction potential and on the measuring electrode. Furthermore when glass-electrodes are used (such as the modern H<sup>+</sup>-ion- and Na<sup>+</sup>-ion electrodes) it must be realized that these are not really specific. Apart from the systematic errors, the accidental errors are such that in measurements of this type the standard deviation of duplicate determination is seldom smaller than 2%.

For these reasons it must be doubted whether it is at present possible to estimate in mixed electrolyte solutions containing protein the activity of any ion with an accuracy better than 3%. This estimate of error is probably an optimistic one. BATES (1954) concludes that electrometric pH measurements cannot truthfully be interpreted in terms of H<sup>+</sup>-ion activity.

#### **Assumptions concerning ion activities in interstitial fluid and plasma**

A study of the literature reveals that for practical purposes the inorganic ions in interstitial fluid are generally considered to be completely dissociated, and to have an activity coefficient of about 0.7 to 0.8. Furthermore although its ionic strength is about 0.16, interstitial fluid is considered sufficiently dilute a solution that ionic strength and valencies fully determine the activity of the various types of ion.

Finally when plasma and interstitial fluid are compared (as in the study of membrane equilibria) it is customary to consider the contribution of proteins to ionic strength to be negligible. This assumption is based on the concept that the effect of protein net charge on ionic strength is that of an equivalent amount of univalent ions. As a consequence of these assumptions the activity coefficient of the various types of filtrable ion

the ions no longer adhere spatially but some electrostatic attraction between the ions will remain until their concentration becomes infinitely low. Conversely the higher their concentration the more the ions will interact the solution will behave less and less like an ideal gas because for each ion the freedom of movement is increasingly restricted. The effect is the same as when there would be a decrease in the number of completely independent particles per unit volume.

One can therefore reconcile the theory of molecular kinetics with the experimental data by considering the hypothetical quantity of completely independent solute particles per unit volume instead of the molar concentrations obtained by chemical analysis. This hypothetical quantity which makes the experimental data fit in with the theory of ideal gas behaviour has been termed by LEWIS the *activity* of a solute. Its relation to the concentration is given by the *activity coefficient*  $f$  where  $f = \text{activity}/\text{concentration}$ . By definition for all solutions  $f = 1.0$  at infinite dilution. From the above considerations it must be concluded that for an electrolyte solution the activity coefficient is related to – and thus a measure for – the interaction between the ions. The stronger the interaction the lower the activity coefficient of the solution. The magnitude of the latter depends therefore on the concentration of the ions, on their valencies, on the temperature of the solution – to name the most important factors.

The large effect of valency is demonstrated by the following data from LEWIS and RANDALL (1923). At 25°C and a molal concentration of 0.1 the value of  $f$  is for NaCl 0.798, for BaCl<sub>2</sub> 0.501 for MgSO<sub>4</sub> 0.166(1).

A measure for the concentrations and valencies of all ions present in a solution is found in the *ionic strength* of that solution, another useful concept of Lewis

The ionic strength ( $\mu$ ) is defined by the equation

$$\mu = \frac{1}{2} \sum C_a Z_a^2 + C_b Z_b^2 + \dots + C_z Z_z^2$$

where  $C$  indicates molar concentrations,  $Z$  valency and the suffix  $a, b, \dots, z$  the various types of ion present in the solution. For plasma the ionic strength is generally calculated at 0.16 on the assumption that in regard to ionic strength the proteins act as univalent ions the number of which is equal to the numerical value of protein net charge.

### Determination of activity coefficient

For solutions containing one or two types of electrolytes the value of  $f$  has been determined experimentally from measurements of freezing point, vapour pressure etc. It is also possible to estimate  $f$  with the aid of equations derived by DEBYE and HÜCKEL. In these equations figure such factors as ionic strength, ion valency, ion diameter, dielectric constant of solvent etc. For dilute solutions (> 0.1 M/L) containing not too many different ions  $f$  can be estimated from ionic strength and valencies by means of a relatively simple equation. At higher concentrations and for polyvalent ions the Debye-Hückel equations become very complicated and the values calculated less accurate.

Until now we have discussed the activity coefficient of a solution. For a single type of ion  $f$  can be estimated in two ways. The first one rests on the assumption proposed by MACINNES that the activities of K<sup>+</sup> and of Cl<sup>-</sup> ions are equal and have the same value as the activity found for a solution of KCl. One can then calculate the activity coefficient for other types of ion by a process of comparison when it is further assumed that in a dilute solution containing various electrolytes the activity coefficient of any ion depends

solely on the ionic strength of the solution. The latter assumption was made by Lewis, who stressed its hypothetical nature and made the restriction that a solution can no longer be considered dilute when its ionic strength increases beyond values of a few hundredths to a few tenths, according to the valency of the ions. It should be noted that Lewis studied solutions containing no macromolecules.

The second way of measuring the activity coefficient of a single type of ion is provided by the principle of the ion-specific electrodes. Essentially these contain the parent atoms of the particular ion for which they are sensitive. When placed in a solution containing this ion an equilibrium will be established between the rates at which ions turn into atoms and vice versa. A change in the activity of the ions in the solution will therefore cause a corresponding change in the rate at which electrons are released from (or taken up by) the electrode. This phenomenon can be measured by incorporating solution and ion-specific electrode in a measuring circuit which contains a potentiometer, a reference electrode in a reference solution, and a liquid junction between the two solutions. If the potentiometer is to indicate correctly the changes in potential at the measuring electrode, then the potentials at the reference electrode and at the junction must have a constant value regardless of the solutions studied. This condition is not easily fulfilled. There are more difficulties.

The need to define activity with the aid of standard solutions indicates that no absolute values for ion activity can be obtained from electrometric measurements. Even the interpretation of changes in potential in terms of changes in ion activity is fraught with theoretical difficulties. Accordingly the number of assumptions necessary is already large in the case of simple salt solutions. When electrolyte solutions containing proteins are studied, the uncertainties become still larger if only because apparently nothing definite is known about the effects which the presence of proteins has such as on the junction potential and on the measuring electrode. Furthermore when glass-electrodes are used (such as the modern H<sup>+</sup>-ion- and Na<sup>+</sup>-ion electrodes) it must be realized that these are not really specific. Apart from the systematic errors, the accidental errors are such that in measurements of this type the standard deviation of duplicate determination is seldom smaller than 2.

For these reasons it must be doubted whether it is at present possible to estimate in mixed electrolyte solutions containing protein the activity of any ion with an accuracy better than 3%. This estimate of error is probably an optimistic one. BATES (1954) concludes that electrometric pH measurements cannot truthfully be interpreted in terms of H<sup>+</sup>-ion activity.

#### Assumptions concerning ion activities in interstitial fluid and plasma

A study of the literature reveals that for practical purposes the inorganic ions in interstitial fluid are generally considered to be completely dissociated, and to have an activity coefficient of about 0.7 to 0.8. Furthermore although its ionic strength is about 0.16 interstitial fluid is considered sufficiently dilute a solution that ionic strength and valencies fully determine the activity of the various types of ion.

Finally when plasma and interstitial fluid are compared (as in the study of membrane equilibria) it is customary to consider the contribution of proteins to ionic strength to be negligible. This assumption is based on the concept that the effect of protein net charge on ionic strength is that of an equivalent amount of univalent ions. As a consequence of these assumptions the activity coefficient of the various types of filtrable ion



the ions no longer adhere spatially but some electrostatic attraction between the ions will remain until their concentration becomes infinitely low. Conversely the higher their concentration the more the ions will interact the solution will behave less and less like an ideal gas because for each ion the freedom of movement is increasingly restricted. The effect is the same as when there would be a decrease in the number of completely independent particles per unit volume.

One can therefore reconcile the theory of molecular kinetics with the experimental data by considering the hypothetical quantity of completely independent solute particles per unit volume instead of the molar concentrations obtained by chemical analysis. This hypothetical quantity which makes the experimental data fit in with the theory of ideal gas behaviour has been termed by Lewis the activity of a solute. Its relation to the concentration is given by the activity coefficient  $f$  where  $f = \text{activity/concentration}$ . By definition for all solutions  $f = 1.0$  at infinite dilution. From the above considerations it must be concluded that for an electrolyte solution the activity coefficient is related to – and thus a measure for – the interaction between the ions. The stronger the interaction the lower the activity coefficient of the solution. The magnitude of the latter depends therefore on the concentration of the ions, on their valencies, on the temperature of the solution to name the most important factors.

The large effect of valency is demonstrated by the following data from Lewis and Randall (1923). At 25°C and a molal concentration of 0.1 the value of  $f$  is for NaCl 0.798, for BaCl<sub>2</sub> 0.501 for MgSO<sub>4</sub> 0.166(1).

A measure for the concentrations and valencies of all ions present in a solution is found in the ionic strength of that solution another useful concept of Lewis.

The ionic strength ( $\mu$ ) is defined by the equation

$$\mu = \frac{1}{2} \sum C_a Z_a^2 + C_b Z_b^2 + \dots + C_n Z_n^2$$

where  $C$  indicates molar concentrations,  $Z$  valency and the suffix  $a, b, \dots, n$  the various types of ion present in the solution. For plasma the ionic strength is generally calculated at 0.16 on the assumption that in regard to ionic strength the proteins act as univalent ions the number of which is equal to the numerical value of protein net charge.

### Determination of activity coefficient

For solutions containing one or two types of electrolytes the value of  $f$  has been determined experimentally from measurements of freezing point, vapour pressure etc. It is also possible to estimate  $f$  with the aid of equations derived by Debye and Hückel. In these equations figure such factors as ionic strength, ion valency, ion diameter, dielectric constant of solvent etc. For dilute solutions (> 0.1 M/L) containing not too many different ions  $f$  can be estimated from ionic strength and valencies by means of a relatively simple equation. At higher concentrations and for polyvalent ions the Debye Hückel equations become very complicated and the values calculated less accurate.

Until now we have discussed the activity coefficient of a solution. For a single type of ion  $f$  can be estimated in two ways. The first one rests on the assumption proposed by MacInnes that the activities of K<sup>+</sup> and of Cl<sup>-</sup> ions are equal and have the same value as the activity found for a solution of KCl. One can then calculate the activity coefficient for other types of ion by a process of comparison when it is further assumed that in a dilute solution containing various electrolytes the activity coefficient of any ion depends

increasing dissociation. The dissociation constant was discussed before. But when cations other than  $H^+$ -ions are studied interest is mostly in the tendency to associate. This tendency is indicated by the reciprocal of the dissociation constant, which is called association constant or binding constant. The latter term results from the fact that physical chemists generally use the term binding without further qualification to indicate complex-binding.

#### Determination of complex-bound ion

When in an electrolyte solution part of a particular type of ion is present in a soluble complex-compound, the concentration obtained by chemical analysis is the sum of the dissociated and the complex-bound (non-dissociated) fraction. Since ionic strength - and hence ion activity - is related to the dissociated ions only the activity of the particular type of ion will be less than its concentration would lead one to expect. In other words, the presence of complex-bound ion manifests itself in a decrease of the activity coefficient of the particular ion below the value calculated with the aid of Debye-Hückel equations on the assumption that all ions are dissociated. In theory therefore the amount of ion that is complex-bound by a certain substance can be determined by comparing the activity coefficients in two solutions which are identical otherwise but one of which contains the particular substance. Among the earliest and most impressive examples of the use of this method for studying ion-protein interaction are the experiments of NORTHROP & KUMTZ (1926, 1928) on ion "binding" by gelatin, and those of HENRIQUES (1929) on the interaction between carbon dioxide derivatives and haemoglobin. In the case of plasma it is rather difficult to measure accurately the activity of a particular type of ion (see previous section). But in the third part of this chapter we will see that the ion distribution observed in experiments in which plasma is subjected to equilibrium dialysis against (synthetic) interstitial fluid can give information concerning complex binding in plasma. That is, provided a number of assumptions is made concerning the ion-activities in plasma (see page 11).

#### THE EFFECT WHICH A HIGH DENSITY OF ELECTROSTATIC CHARGE ON THE SURFACE OF THE PROTEIN MOLECULES WILL HAVE ON THE IONS IN THE SURROUNDING SOLUTION

We have assumed that the electrostatic interaction between the ions of a dissociated salt is non-specific, and that their activity can be predicted from the valencies of the ions and the ionic strength of the solution, provided the latter is sufficiently dilute. The assumptions are based on the concept of a homogenous distribution of electrostatic charge throughout the solution. This concept is wrong when the solution contains protein molecules with a high density of electrostatic charges in certain areas of their surface. In that case ions carrying an opposite charge accumulate around the areas of high charge, and the higher the valency of the counter ions the stronger they will be attracted. In this connection it must be realised that local accumulations of dissociated groups may exist while the net charge of the protein is zero. If the density of the surface charge becomes sufficiently high these counter ions will be much more restricted in their freedom of movement - *i.e.* in their activity - than the ionic strength, calculated for the solution as a whole, would lead one to expect. This extra decrease in activity

are considered to be equal in plasma and in interstitial fluid, as long as the proteins interact with the filtrable ions in solution in the manner of the ions of a fully dissociated salt. At the ionic strength of plasma this electrostatic interaction is considered to be non specific.

## INTERACTION BETWEEN NEUTRAL MOLECULES AND IONS

### Complex-binding

In contrast to the long-range effects of electrostatic forces, the forces acting between a neutral molecule and an ion are very strong when the two are at a distance of a few Å, but their strength decreases with extreme rapidity when the distance increases. In this type of interaction we need therefore distinguish only two possible states. Either the ion is associated with and attached to the molecule or it is not. In the latter case the interaction between molecule and ion may be neglected in comparison to the electrostatic interactions between the ions. The ammonia molecule is an example of a neutral molecule which can associate with an ion

The nitrogen atom shares 3 of the 5 electrons present in its outer electron shell each with one H-atom. The two remaining electrons ( lone pair ) tend to associate with cations which are one or two electrons short, such as an  $H^+$  ion



Ammonia is therefore a proton acceptor (a base), and since the ammonia molecule is neutral the result of association is a positively charged ammonium ion. The same applies to the amino group.

This type of ion formation is an example of association by coordination or *complex binding*. Molecules containing a central atom with one or more lone pairs of electrons (nitrogen oxygen sulphur) tend to form such coordination compounds.

By its nature complex binding allows for selective attraction of ions, and this selectivity can become very pronounced indeed when several such groups are suitably arranged spatially. Such arrangements partly explain the high affinity of certain molecular structures for ions such as  $Zn^{2+}$ ,  $Cu^{2+}$ ,  $Mn^{2+}$  and  $Fe^{2+}$ . That spatial arrangement can induce a certain selectivity even in electrostatic attraction is demonstrated by the affinity of citrate ions for  $Ca^{2+}$  ions.

In contrast to the univalent cations the bivalent  $Ca^{2+}$  ion fits between two of the three dissociated carboxyl groups of citrate and is so firmly incorporated in the citrate molecule that both the two carboxyl groups and the  $Ca^{2+}$  ion cease to contribute to the ionic strength of the solution (HASTINGS *et al.* 1934).

Associations which result in the formation of similar ringlike structures are indicated by the term *chelation*.

### Association or binding constant

In the case of complex binding the interaction between molecule and ion practically ceases to exist when the two dissociate. Complex binding can therefore be described in terms of the Mass Law and an equilibrium constant calculated. When the interest is in the tendency to dissociate (as is the case with the acid complex-compounds  $-COOH$  and  $-H^+NH_2$ ) the equation is written so that the value of the constant increases with

increasing dissociation. The dissociation constant was discussed before. But when cations other than H<sup>+</sup>-ions are studied interest is mostly in the tendency to associate. This tendency is indicated by the reciprocal of the dissociation constant, which is called association constant or binding constant. The latter term results from the fact that physical chemists generally use the term binding without further qualification to indicate complex-binding.

#### Determination of complex-bound ion

When in an electrolyte solution part of a particular type of ion is present in a soluble complex-compound, the concentration obtained by chemical analysis is the sum of the dissociated and the complex-bound (non-dissociated) fraction. Since ionic strength – and hence ion activity – is related to the dissociated ions only the activity of the particular type of ion will be less than its concentration would lead one to expect. In other words, the presence of complex-bound ion manifests itself in a decrease of the activity coefficient of the particular ion below the value calculated with the aid of Debye-Hückel equations on the assumption that all ions are dissociated. In theory therefore the amount of ion that is complex bound by a certain substance can be determined by comparing the activity coefficients in two solutions which are identical otherwise but one of which contains the particular substance. Among the earliest and most impressive examples of the use of this method for studying ion-protein interaction are the experiments of NORTHBOROUGH & KUNITZ (1926-1928) on ion binding by gelatin, and those of HENRIQUES (1929) on the interaction between carbon dioxide derivatives and haemoglobin. In the case of plasma it is rather difficult to measure accurately the activity of a particular type of ion (see previous section). But in the third part of this chapter we will see that the ion distribution observed in experiments in which plasma is subjected to equilibrium dialysis against (synthetic) interstitial fluid can give information concerning complex-binding in plasma. That is, provided a number of assumptions is made concerning the ion-activities in plasma (see page 11).

#### THE EFFECT WHICH A HIGH DENSITY OF ELECTROSTATIC CHARGE ON THE SURFACE OF THE PROTEIN MOLECULES WILL HAVE ON THE IONS IN THE SURROUNDING SOLUTION

We have assumed that the electrostatic interaction between the ions of a dissociated salt is non-specific, and that their activity can be predicted from the valencies of the ions and the ionic strength of the solution, provided the latter is sufficiently dilute. The assumptions are based on the concept of a homogenous distribution of electrostatic charge throughout the solution. This concept is wrong when the solution contains protein molecules with a high density of electrostatic charges in certain areas of their surface. In that case ions carrying an opposite charge accumulate around the areas of high charge, and the higher the valency of the counter ions the stronger they will be attracted. In this connection it must be realised that local accumulations of dissociated groups may exist while the *net* charge of the protein is zero. If the density of the surface charge becomes sufficiently high these counter ions will be much more restricted in their freedom of movement – i.e. in their activity – than the ionic strength calculated for the solution as a whole, would lead one to expect. This extra decrease in activity

is then not the result of complex binding and cannot therefore be described by the stoichiometric relations of the Mass Law. But, especially in solutions such as plasma, it is difficult to distinguish between true complex binding and the type of strong electrostatic attraction mentioned above. In the latter case also a certain selectivity exists in that ion characteristics such as valency, diameter of hydrated ion etc. modify the strength of the attraction.

This adsorption of small ions by strong electrostatic forces on a surface is an old concept. It explains quite a number of phenomena in colloid chemistry (see for instance the work of Bungenberg de Jong and co-workers on the association colloids - BUNGENBERG DE JONG *et al* in *Colloid Science* vol II 1949). The concept got somewhat out of fashion in the American literature on protein chemistry, but in recent years the experience with the synthetic ion exchangers - which have a very high density of electrostatic charge - has been a reminder of the importance of adsorption phenomena in the vicinity of accumulated electrostatic charge.

It is customary in calculations of the ionic strength of plasma either to neglect the charge on the proteins or to consider its effect to be no more than that of a number of univalent ions. As a consequence an extra decrease in ion-activity due to the unsuspected presence of areas with a high density of electrostatic charge will be interpreted as indicating true complex binding.

#### INTERACTION BETWEEN PROTEIN AND WATER MOLECULES

Points of charge on protein molecules attract water molecules in the same manner as do small cations and anions and there are indications that up to two molecules of water are bound per point of charge (WAUGH 1954). Although it is the general custom to calculate all concentrations per unit weight of water (obtaining the latter by drying a sample of protein solution to constant weight) it might be asked whether this value should not be corrected for the water bound to cationic and anionic substances in much the same manner as the ion-concentration is corrected to obtain ion activity (see for instance GROLLMAN 1931). But the difficulties involved are considerable, one being how to distinguish between an increase in the activity of solute and a decrease in activity of water. For the present to express the concentration of solute per unit weight of water remains the best quantitative description of the components of a solution.

#### INTERACTION BETWEEN THE VARIOUS PROTEIN MOLECULES

Proteins with different IEP might be expected to interact with each other if the pH value of the solution is in the intermediate range. But for the plasma proteins we have found no data concerning this effect. In experiments with plasma it is generally neglected.

#### DEFINITION OF THE TERM BINDING DISTINCTION IN 'SALT TYPE' AND 'COMPLEX TYPE'

From the previous sections it will be clear that in plasma the interactions between proteins and small ions must range from the typical salt-type of interaction in which the ion is fully dissociated, to the typical complex-binding in which the ion is no longer

dissociated. Between these extremes a variety of interactions must exist which are of an intermediate type in that they result in a more or less pronounced restriction of ion movement. For practical purposes it is necessary to reduce the number of possible interactions to a few main types. The most convenient simplification is to distinguish two types according to whether or not the activity of the ion decreases below the value which would be calculated from the ionic strength of the solution and the valencies of the ions with the aid of the Debye Hückel equations.

In this study the term *binding* will be used to indicate any attraction between small, i.e. filtrable ions and proteins regardless of the nature and the strength of the attracting forces. Two types will be distinguished.

(a) The binding will be considered to be of the *salt-type* when in the presence of the protein the activity coefficient of the filtrable ion under consideration is not different from that in a solution of equal ionic composition and ionic strength but containing no protein. In this type of binding the ion is considered to be dissociated from the protein. The (electrostatic) attraction between protein and ion is in this case essentially non-specific and only demands that the charge on the protein is - statistically - balanced by an equivalent charge of opposite sign in the surrounding solution.

(b) The binding will be considered to be of the *complex-type* when it leads to a decrease in the activity coefficient of the filtrable ion below the value expected in an electrolyte solution which is identical but for the presence of protein. This decrease in activity coefficient can be the result of association of the ion because of true complex-binding or chelation, or it may be the expression of an inordinate restriction of ion-movement as a result of strong electrostatic attraction. In a complicated solution such as plasma these modes of interaction cannot at present be distinguished from each other.

The above resolution of all possible interactions in two types is at the back of most discussions on ion-protein binding. Apparently however the pragmatic nature of the distinction is seldom realised. As a consequence decreases of ion activity below the value expected from ionic strength are interpreted as indicative of true complex-binding. Whereas this may be sufficiently near the truth for those cations which show an excessive tendency to complex-formation such as  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$  it must be doubted whether it is correct in the case of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  and in the case of the univalent cations. If strong electrostatic charges on the protein molecules severely depress the activity of the counter ions but the latter remain dissociated, calculations based on the assumption that binding is either of the salt-type or of the complex-type, will automatically result in estimating a (hypothetical) fraction of the ions as truly complex-bound and the remainder as fully dissociated in the manner of the ions of a salt. The interpretation of the values thus obtained in terms of a definite number of binding sites and their use for calculating association constants must be viewed with suspicion in the case of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  and certainly in the case of  $\text{Na}^{+}$  and  $\text{K}^{+}$ .

All this does not detract from the practical value of the schematic distinction of two types of binding. It is very useful when analysing the differences in behaviour of the various ions towards the proteins. But in view of the numerous assumptions made, values calculated for ion bound in respectively a salt-type or a complex type manner have a relative rather than an absolute significance.

This restriction must be kept in mind when reading the following pages.

is then not the result of complex binding and cannot therefore be described by the stoichiometric relations of the Mass Law. But, especially in solutions such as plasma, it is difficult to distinguish between true complex binding and the type of strong electrostatic attraction mentioned above. In the latter case also a certain selectivity exists in that ion characteristics such as valency, diameter of hydrated ion etc. modify the strength of the attraction.

This adsorption of small ions by strong electrostatic forces on a surface is an old concept. It explains quite a number of phenomena in colloid chemistry (see for instance the work of Bungenberg de Jong and co-workers on the association colloids - BUNGENBERG DE JONG *et al.* in *Colloid Science* vol. II 1949). The concept got somewhat out of fashion in the American literature on protein chemistry, but in recent years the experience with the synthetic ion exchangers - which have a very high density of electrostatic charge - has been a reminder of the importance of adsorption phenomena in the vicinity of accumulated electrostatic charge.

It is customary in calculations of the ionic strength of plasma either to neglect the charge on the proteins or to consider its effect to be no more than that of a number of univalent ions. As a consequence an extra decrease in ion activity due to the unsuspected presence of areas with a high density of electrostatic charge will be interpreted as indicating true complex binding.

#### INTERACTION BETWEEN PROTEIN AND WATER MOLECULES

Points of charge on protein molecules attract water molecules in the same manner as do small cations and anions and there are indications that up to two molecules of water are bound per point of charge (WAUGH 1954). Although it is the general custom to calculate all concentrations per unit weight of water (obtaining the latter by drying a sample of protein solution to constant weight) it might be asked whether this value should not be corrected for the water bound to cationic and anionic substances in much the same manner as the ion-concentration is corrected to obtain ion activity (see for instance GROLLMAN 1931). But the difficulties involved are considerable, one being how to distinguish between an increase in the activity of solute and a decrease in activity of water. For the present to express the concentration of solute per unit weight of water remains the best quantitative description of the components of a solution.

#### INTERACTION BETWEEN THE VARIOUS PROTEIN MOLECULES

Proteins with different IEP might be expected to interact with each other if the pH value of the solution is in the intermediate range. But for the plasma proteins we have found no data concerning this effect. In experiments with plasma it is generally neglected.

#### DEFINITION OF THE TERM BINDING DISTINCTION IN SALT TYPE AND COMPLEX TYPE

From the previous sections it will be clear that in plasma the interactions between proteins and small ions must range from the typical salt-type of interaction in which the ion is fully dissociated to the typical complex-binding in which the ion is no longer

sociated filtrable anion. According to whether respectively more cation or more anion is bound in a salt-type manner the protein net charge is respectively negative or positive.

It must be stressed that the above definition of net charge differs from the one given in the first part of this chapter where net charge was defined as the arithmetic sum of the positive and the negative charges on an amphoterie substance. The latter definition is the more correct one theoretically. But since in a solution such as plasma the net charge of the proteins can only be measured indirectly from its effect on the surrounding dissociated filtrable cations and anions, the definition accepted for the purpose of our study has practical advantage. Since the electrostatic attraction responsible for the salt-type of binding cannot be described in terms of the Mass Law the values calculated for protein net charge in the indirect manner described above does not necessarily represent an identical number of charged ionogenic groups on the protein molecules.

#### Relation between NCE and negative net charge in plasma

In the physiological pH range more cation than anion is bound by plasma protein. In the case of plasma we will therefore have to use the terms 'net cation equivalency' and 'negative net charge'. The relation between the two can be described as follows:  $(NCE) = (\text{negative net charge}) + (\text{complex bound cation}) - (\text{complex bound anion})$ , all expressed in equivalent concentrations.

### THE EFFECT OF PROTEIN ON THE DISTRIBUTION OF FILTRABLE IONS ACROSS A SEMIPERMEABLE MEMBRANE AND THE USE OF THIS EFFECT IN THE STUDY OF PROTEIN BOUND ION

#### MEMBRANE EQUILIBRIA

When two identical electrolyte solutions, containing filtrable ions only, are separated by a semipermeable membrane the osmotic pressure of the solution and the activity of each ion will be equal on both sides of the membrane. The ratio of the activities is therefore 1.000. If then a non-filtrable ionic substance, such as protein, is added to one of the two solutions the osmotic and electrostatic equilibrium is disturbed. After a while equilibrium will be re-established by water molecules moving through the semipermeable membrane into the protein solution, and by filtrable ions moving in the opposite direction. In the new state of equilibrium the increase in hydrostatic pressure of the protein containing solution balances the combined osmotic effect of the presence of protein and of the redistribution of filtrable ions. At the same time the unilateral presence of non-filtrable electrostatic charge is compensated for by an unequal activity of the filtrable ions in the two solutions. The activity ratios therefore differ from 1.000. Since for thermodynamical reasons the product of the activities of filtrable cation and filtrable anion must, at equilibrium, be the same in both solutions, while simultaneously electroneutrality should exist in each solution, the change in activity ratio is in opposite directions for cations and for anions. Sign and magnitude of the non-filtrable electrostatic charge (net charge in case of amphoterie substances) determine in what direction, and how much, the activity ratios will differ from 1.000. The unequal distribution of the filtrable ions will also manifest itself in a potential difference between the two solutions (membrane potential).

Donnan has been the first to demonstrate these characteristics of membrane equilibria.



DEFINITION OF THE TERMS PROTEIN NET ION EQUIVALENCY AND PROTEIN NET CHARGE AS THEY ARE USED IN THIS STUDY - INTRODUCTION OF THE TERM NET CATION EQUIVALENCY' (NCE) INSTEAD OF THE TERM BASE BINDING POWER

The resultant or 'net effect of proteins on the cation-anion diagram of an electrolyte solution

In an electrolyte solution protein contributes to both the cationic and anionic properties of the solution. The net effect of this contribution can be measured by subtracting from one another the experimentally determined sum concentrations of the filtrable cations and the filtrable anions. The value thus obtained must be equal to the difference between the total amount of protein bound cation and the total amount of protein-bound anion regardless of the nature of the binding. When both cations and anions are bound in a salt type manner only then the difference between their respective sum concentrations relates to fully dissociated ions and it will therefore be equal to protein net charge. But when cation and/or anion is bound in a complex type manner as well, then the difference between the sum concentrations of filtrable cation and of filtrable anion includes non-dissociated ion and is therefore not equal to protein net charge.

For plasma, where the resultant effect of protein is that of an anion, the difference between the concentration of filtrable cations and that of filtrable anions as found by chemical analysis has been termed base binding power by Van Slyke and co-workers. Later in order to avoid the ambiguous term base MANERY (1954) introduced the term cation equivalency.

Because no clear distinction was made between salt and complex type of ion-protein binding 'base binding power' is generally considered as being identical with net charge. Furthermore, the terms base binding power and cation equivalency fail to stress the net character of the quantity to which they refer. In this study therefore the following terms will be used.

*Net ion equivalency of the proteins* This is the difference between protein-bound cation and protein-bound anion regardless of the nature of the binding. It is measured by subtracting from one another the concentrations of the filtrable cations and the filtrable anions as obtained by chemical analysis. According to whether the proteins bind more cation or more anion we will use the terms net cation equivalency or net anion equivalency. Since in plasma the proteins bind cations in excess of anions we will use the term *net cation equivalency*, which will be abbreviated to NCE. This term is synonymous with the term base binding power. The terms *cation (anion) equivalency* will indicate the amount of filtrable cation (anion) bound by protein regardless of the nature of the bond. By definition

$(NCE) = (\text{cation equivalency}) - (\text{anion equivalency})$  all expressed in equivalent concentration

*Net charge of the proteins* This is quantitatively equal to the difference between the equivalent amounts of cation and of anion that are protein-bound in a salt-type of manner. By definition therefore protein net charge has a value equivalent to the difference between the concentration of fully dissociated filtrable cation and that of fully dis-

sociated filtrable anion. According to whether respectively more cation or more anion is bound in a salt type manner the protein net charge is respectively negative or positive.

It must be stressed that the above definition of net charge differs from the one given in the first part of this chapter where net charge was defined as the arithmetic sum of the positive and the negative charges on an amphoteric substance. The latter definition is the more correct one theoretically. But since in solution such as plasma the net charge of the proteins can only be measured indirectly from its effect on the surrounding dissociated filtrable cations and anions, the definition accepted for the purpose of our study has practical advantage, since the electrostatic attraction responsible for the salt-type of binding cannot be described in terms of the Mass Law the value calculated for protein net charge in the indirect manner described above does not necessarily represent an identical number of charged ionogenic groups on the protein molecules.

#### Relation between NCE and negative net charge in plasma

In the physiological pH range more cation than anion is bound by plasma protein. In the case of plasma we will therefore have to use the terms 'net cation equivalency' and 'negative net charge'. The relation between the two can be described as follows: (NCE) = (negative net charge) + (complex-bound cation) - (complex bound anion), all expressed in equivalent concentrations.

### THE EFFECT OF PROTEIN ON THE DISTRIBUTION OF FILTRABLE IONS ACROSS A SEMIPERMEABLE MEMBRANE AND THE USE OF THIS EFFECT IN THE STUDY OF PROTEIN-BOUND ION

#### MEMBRANE EQUILIBRIA

When two identical electrolyte solutions, containing filtrable ions only are separated by a semipermeable membrane the osmotic pressure of the solution and the activity of each ion will be equal on both sides of the membrane. The ratio of the activities is therefore 1.000. If then a non-filtrable ionic substance, such as protein, is added to one of the two solutions the osmotic and electrostatic equilibrium is disturbed. After a while equilibrium will be re-established by water molecules moving through the semipermeable membrane into the protein solution, and by filtrable ions moving in the opposite direction. In the new state of equilibrium the increase in hydrostatic pressure of the protein containing solution balances the combined osmotic effect of the presence of protein and of the redistribution of filtrable ions. At the same time the unilateral presence of non-filtrable electrostatic charge is compensated for by an unequal activity of the filtrable ions in the two solutions. The activity ratios therefore differ from 1.000. Since for thermodynamical reasons the product of the activities of filtrable cation and filtrable anion must, at equilibrium, be the same in both solutions, while simultaneously electroneutrality should exist in each solution, the change in activity ratio is in opposite directions for cations and for anions. Sign and magnitude of the non-filtrable electrostatic charge (net charge in case of amphoteric substances) determine in what direction and how much, the activity ratios will differ from 1.000. The unequal distribution of the filtrable ions will also manifest itself in a potential difference between the two solutions (membrane potential).

DOUGAN has been the first to demonstrate these characteristics of membrane equilibria

DEFINITION OF THE TERMS PROTEIN 'NET ION EQUIVALENCY' AND PROTEIN NET CHARGE AS THEY ARE USED IN THIS STUDY - INTRODUCTION OF THE TERM 'NET CATION EQUIVALENCY (NCE)' INSTEAD OF THE TERM BASE BINDING POWER

The resultant or 'net' effect of proteins on the cation-anion diagram of an electrolyte solution

In an electrolyte solution protein contributes to both the cationic and anionic properties of the solution. The net effect of this contribution can be measured by subtracting from one another the experimentally determined sum concentrations of the filtrable cations and the filtrable anions. The value thus obtained must be equal to the difference between the total amount of protein bound cation and the total amount of protein bound anion regardless of the nature of the binding. When both cations and anions are bound in a salt type manner only then the difference between their respective sum concentrations relates to fully dissociated ions and it will therefore be equal to protein net charge. But when cation and/or anion is bound in a complex type manner as well, then the difference between the sum concentrations of filtrable cation and of filtrable anion includes non-dissociated ion and is therefore not equal to protein net charge.

For plasma, where the resultant effect of protein is that of an anion the difference between the concentration of filtrable cations and that of filtrable anions as found by chemical analysis has been termed base binding power by Van Slyke and co-workers. Later in order to avoid the ambiguous term base MANERY (1954) introduced the term cation equivalency.

Because no clear distinction was made between salt and complex type of ion-protein binding base binding power is generally considered as being identical with net charge. Furthermore the terms base binding power and cation equivalency fail to stress the net character of the quantity to which they refer. In this study therefore the following terms will be used.

*Net ion equivalency of the proteins* This is the difference between protein bound cation and protein bound anion regardless of the nature of the binding. It is measured by subtracting from one another the concentrations of the filtrable cations and the filtrable anions as obtained by chemical analysis. According to whether the proteins bind more cation or more anion we will use the terms net cation equivalency or net anion equivalency. Since in plasma the proteins bind cations in excess of anions we will use the term *net cation equivalency* which will be abbreviated to NCE. This term is synonymous with the term base binding power. The terms *cation (anion) equivalency* will indicate the amount of filtrable cation (anion) bound by protein regardless of the nature of the bond. By definition

$(NCE) = (\text{cation equivalency}) \text{ minus } (\text{anion equivalency})$  all expressed in equivalent concentration

*Net charge of the proteins* This is quantitatively equal to the difference between the equivalent amounts of cation and of anion that are protein-bound in a salt type of manner. By definition therefore protein net charge has a value equivalent to the difference between the concentration of fully dissociated filtrable cation and that of fully dis-

out that values calculated with the aid of equations which are based on the assumptions mentioned above can only have a relative significance.

The determination of the Donnan ratio for the equilibrium plasma-synthetic interstitial fluid as observed in equilibrium dialysis experiments

The concentration ratios for the univalent cations and anions observed in experiments of this type (see chapter II) suggest that for normal plasma the value of the Donnan ratio must be somewhere between 0.93 and 0.98. If the Donnan ratio is to be of any use in determining whether and if so to what extent, the univalent cations and anions in plasma are complex-bound, then the value of the Donnan ratio has to be known within 0.005 as we will see in the discussion of our own experiments.

Measurement of the membrane potential (from which the Donnan ratio can be calculated by means of one of the equations derived by Donnan) is not at present sufficiently accurate because of systematic and accidental errors.

The following example only serve to show the accuracy needed. If the Donnan ratio were 0.950 the membrane potential should be 1.31 mV (at 38°C). For a Donnan ratio of 0.960 the relevant equation indicates a potential of 1.05 mV. One will be hard put to detect such a small potential difference with certainty. The possibility of large systematic errors is indicated by the recent study of SALMUND (1961). He measured among other things the potential difference between normal human serum and its ultrafiltrate. The average value was found to be about 9 mV which would correspond with a Donnan ratio of about 0.69. The value is absurd in view of the fact that in the same experiments the concentration ratio of Na was on the average 0.92.

The relative inaccuracy is still greater when the Donnan ratio is calculated from the ion activities as measured in plasma respectively in interstitial fluid by means of ion-specific electrodes. In this case the errors of two measurements are summated. The errors to be expected have been indicated already (page 11).

Some authors notably GREENE & POWER (1931), assumed that in physiological pH values none of the plasma Cl<sup>-</sup> was bound in a complex-type manner. The concentration ratio of Cl<sup>-</sup> was therefore accepted as being equal to the Donnan ratio. But the results of recent experiments with purified albumin (to be discussed in the next part of this chapter) suggest that part of the plasma Cl<sup>-</sup> is after all complex-bound.

VAN SLYKE *et al.* calculated  $r$  from data which at first sight are obtained with relative ease. In their masterly treatise on the factors controlling the electrolyte and water distribution in the blood (1923) they derived a number of equations among which one defining the relation between Donnan ratio, protein net charge and concentration ratio of ion for the equilibrium serum-interstitial fluid. But the authors neglected the fact that a certain amount of the ions present in plasma is complex-bound by protein and the equations must therefore be reconsidered.

An additional difficulty is the fact that only the "base binding power" as determined by Van Slyke *et al.* in 1928 is available for calculating protein net charge. Apart from the fact that it remains to be seen whether protein net charge can be obtained from "base binding power" it will be shown (chapter II) that the value found by Van Slyke *et al.* can no longer be accepted unless substantiated by fresh experimental evidence.

In view of all this it is not surprising that even for the *in vitro* equilibrium between plasma (or serum) and synthetic interstitial fluid the precise value of the Donnan ratio remains a matter of conjecture (MÄNERY 1954).

(DONNAN & HARRIS 1911) With the aid of thermodynamic considerations published by GIBBS DONNAN could derive equations which describe the osmotic and electrostatic phenomena existing across a semipermeable membrane (DONNAN 1911 1924) For the derivation of the fundamental equations the reader is referred to Donnan's publications. Donnan expressly stated that these equations are only valid for equilibrium conditions, and that - since they are based on thermodynamical considerations - the equations are concerned with activities not with concentrations

LOEB (1922) has been the first to apply the Donnan theory to biological problems. Since then many have followed who made use of it in the study of osmotic or electrostatic phenomena. Since in this study we are concerned with the latter we will neglect the osmotic effect of membrane equilibrium. This is permissible because we will only consider equilibrium conditions, i.e. conditions in which osmotic and electrostatic equilibrium exists simultaneously. The two phenomena can then be considered separately

#### THE DONNAN RATIO AND ITS USEFULNESS FOR ANALYSING ION-PROTEIN INTERACTION

##### The Donnan ratio

When plasma is in equilibrium with interstitial fluid through a semipermeable membrane, we can consider plasma as interstitial fluid to which protein has been added. According to the theory of Donnan the ratio of the ion activities will for this equilibrium be defined by the following equation

$$r = \frac{f[H^+]_o}{f[H^+]_i} = \frac{f[Na^+]_o}{f[Na^+]_i} = \frac{f[K^+]_o}{f[K^+]_i} = \frac{\sqrt{f[Ca^{2+}]_o}}{\sqrt{f[Ca^{2+}]_i}} = \frac{f[Cl^-]_i}{f[Cl^-]_o} = \frac{f[HCO_3^-]_i}{f[HCO_3^-]_o}$$

where the suffix *i* indicates the protein containing solution ('inside'), the suffix *o* the non-protein solution ('outside')

The symbol *r* is called the Donnan ratio. Its magnitude depends for any given equilibrium on the protein concentration and the total concentration of filtrable electrolytes (see later). For the present the most important aspect of the Donnan ratio is that with its aid the activity ratio can be calculated for each of the filtrable ions. Conversely we need only measure the activity ratio for one of the ions to know the Donnan ratio

##### The relation between Donnan ratio and concentration ratios

In the case of the equilibrium between plasma and interstitial fluid the Donnan theory can be made to apply to concentration ratios if it is assumed that plasma and interstitial fluid have the same ionic strength, and therefore the same activity coefficients for the dissociated ions (see page 11). The concentration ratio of an ion will then equal its activity ratio and hence the Donnan ratio, provided that the plasma proteins only interact with the particular ion in a salt type manner. When the proteins bind part of the ion in a complex type manner the concentration ratio of the ion will differ from the Donnan ratio and consequently from the concentration ratios of the ions which are bound in a salt-type manner only.

The above mentioned simplification has been generally made in previous studies concerning the ion distribution between plasma and interstitial fluid. Since no workable alternative presents itself we will make the same simplification. But it should be pointed

the amount of a particular type of filtrable cation or anion associated with protein in salt-type manner are necessarily subject to the same restriction. Since at physiological pH the net charge of the serum proteins has a negative sign, the result will be that only cations will appear to be protein-bound in a salt-type manner and calculations concerning the extent of this type of binding will only reveal the amount of cation bound in excess of anion. This restriction does not apply to the calculation of complex-bound cation or anion, since these are not subject to the law of electroneutrality.

Fig. 2 is also *errata* in that no filtrable complex-bound substances are shown, although plasma contains small amount of such compounds (Ca-citrate and possibly some Ca-phosphate). Since these are not subject to the law of electroneutrality their concentration is the same on both sides of the membrane and before we can apply the equations which are to follow the total concentration of the cation or anion involved should be corrected for the amount present in such filtrable complex-compounds.

However since in our dialysis experiments to be reported later the total concentration of these substances must have been less than 0.1 m mole per litre we have followed previous authors in neglecting their presence.

### Calculation of Donnan ratio

In the outward compartment depicted in fig. 2 both cations and anions are present as freely filtrable ion only ( $\text{Cat}^+_{\text{f}}, \text{An}^-_{\text{f}}$ ). In the protein-containing compartment anion is also present as freely filtrable anion only ( $\text{An}^-_{\text{f}}$ ), but cation is present both as filtrable ion ( $\text{Cat}^+_{\text{f}}$ ) and complex-bound by protein ( $\text{Cat}^+_{\text{Pr}}$ ). The greatest part of  $\text{Cat}^+_{\text{f}}$  is balanced by  $\text{An}^-_{\text{f}}$ , the remainder by the negative charges on the protein molecules ( $\text{Pr}^-$ ). All quantities are expressed in equivalent concentration and it must be realised that  $\text{Pr}^-$  is equivalent to the amount of univalent cation balancing the anionic groups on the protein molecules.

Since the Donnan ratio is only affected by the activity of the ions in solution, we find

$$r = \frac{f[\text{Cat}^+]_{\text{f}}}{f[\text{Cat}]_{\text{f}}} = \frac{f[\text{An}]_{\text{f}}}{f[\text{An}]_{\text{p}}}$$

And since we have assumed ionic strength to be equal in both solutions

$$r = \frac{[\text{Cat}^+]_{\text{f}}}{[\text{Cat}^+]_{\text{f}}} = \frac{[\text{An}]_{\text{f}}}{[\text{An}]_{\text{p}}} \quad (1)$$

In the situation depicted in fig. 2 the analytically determined ratio of the cation, *i.e.* the concentration ratio (hence forward to be indicated by the symbol  $R_{\text{cat}}$ ), will be smaller than  $r$ . This is evident from the following equation

$$R_{\text{cat}} = \frac{[\text{Cat}]_{\text{f}}}{[\text{Cat}]} = \frac{[\text{Cat}]_{\text{f}}}{[\text{Cat}^+]_{\text{f}} + [\text{Cat Pr}]} < r \quad (2)$$

Acknowledging the fact that  $[\text{Cat}^+]_{\text{f}} = [\text{Cat}]_{\text{f}}$  because the ions are considered to be fully dissociated in the absence of protein, we find by substitution from (1) and (2)

$$r = \frac{[\text{Cat}]_{\text{f}} \times R_{\text{cat}}}{[\text{Cat}]_{\text{f}} - [\text{Cat Pr}] \times R_{\text{cat}}} \quad (3)$$

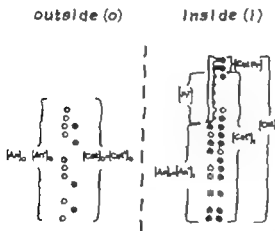
As in our opinion the approach made by Van Slyke and associates appears at present to be the most promising one, we will reconsider their equations for calculating the value of  $r$  while making allowance for the presence of complex bound ion. Once the Donnan ratio is known the extent to which an ion is complex bound can be calculated from its concentration ratio by means of relatively simple equations. This was demonstrated already by Northrop and Kunitz.

For what follows we are greatly indebted to VAN SLYKE *et al* (1923), NORTHROP & KUNITZ (1925 1926) and GREENE & POWER (1931) whose publications have contributed largely to our understanding.

#### EQUATIONS FOR CALCULATING THE DONNAN RATIO AND THE QUANTITY AND QUALITY OF PROTEIN BOUND ION

##### Schematic representation of equilibrium between serum and interstitial fluid

The scheme to be used in the following discussion (fig. 2) is a simplified representation of serum in equilibrium with interstitial fluid across a semipermeable membrane. It is simplified in that only one type of filtrable cation and anion is represented and in that



LEGEND: Cation, Anion, Protein, determined by chemical analysis, present in solution, complex bound by protein, sum of charges groups on protein.

Fig. Schematic representation of equilibrium existing between serum and interstitial fluid across semipermeable membrane.

protein is represented as carrying a negative charge only. The first simplification is not a serious one, because no matter how many different cations and anions are present, individually they have to conform to the same laws. The second simplification will not affect the calculation of the Donnan ratio as the latter only depends on the net charge of the proteins. But at the same time this implies that a macromolecule carrying an identical amount of negative and of positive charges will not influence the partition of ions across a semipermeable membrane. In this respect it cannot be distinguished from a macromolecule which carries no charge at all. It follows that, in as far as negatively charged ionogenic groups on the protein molecules are compensated by positively charged groups on the same molecules, they cannot be detected by studying the distribution of filtrable ions across a semipermeable membrane. All conclusions concerning

ed that owing to the assumption of equal ionic strengths for plasma and for interstitial fluid no activity coefficients appear in the equation. In the second part of this chapter it has been argued that the cations balancing the negatively charged sites on the protein surface may have lower activity coefficient than the other ions in the solution. In that case different activity coefficients would have to be inserted in equation (6) for  $[An^-]_b$  and for  $[Pr^-]$  in order to obtain the correct Donnan ratio. Neglect of the activity coefficients will lead to an erroneously low value for  $r$ . Conversely when  $r$  is known and equation (6) is used to calculate the negative net charge,  $[Pr^-]$ , the latter will be calculated too low. Although therefore in equation (6) the error due to the possible presence of  $AnPr$  is minimized, we have introduced with  $Pr^-$  a new difficulty.

We have now three equations from which in theory  $r$  can be obtained: equation (3) or (5) can be used should it be known which fraction of cation or anion is complex-bound by protein; equation (6) is available in case the concentration of filtrable anion in the protein-containing solution is known as well as protein net charge.

#### Calculation of quality and quantity of protein-bound ion

Since  $R_{cat}$  respectively  $R_{an}$  are related to  $r$  and to  $CatPr$  respectively  $AnPr$  (see equations (3) and (5)), the complex bound fraction of an ion can be calculated when both its concentration ratio and the Donnan ratio are known. The equations differ somewhat according to whether the ion is univalent or bivalent.

We will first consider the univalent cation in fig. 2. By chemical analysis we find  $[Cat]_b$  and  $[Cat]_f$ . The difference between these two values to be indicated as  $\Delta Cat$  is related to the total amount of cation bound by protein. But whereas the latter is equal to  $[CatPr] + [Pr^-]$ , it will be seen that  $\Delta Cat = [Cat]_f - [Cat]_b = [CatPr] + [Cat]_f - [Cat^+]_b$ , and  $\Delta Cat$  therefore underestimates the total amount of protein-bound cation. From fig. 2 it will be evident that the underestimate is equal to  $[An]_b - [An]_f$ , which according to equation (1) can also be written as  $(1 - r)[An]_b$ . And since  $[An]_b$  must be equal to  $[Cat^+]_b$  we find that

$$\text{protein-bound univalent cation} = \Delta Cat + (1 - r)[Cat]_b \quad (7)$$

Provided  $r$  is known we should therefore be able to calculate the total amount of cation which is protein-bound. However as we discussed already the amount thus calculated is a minimum value since  $Pr^-$  stands in fact for the negative net charge. The total negative charge of the proteins may be considerably larger but the difference escapes detection, since it is compensated by the positive charge of the proteins which we have no way of measuring. The amount of anion which is protein-bound in a salt-type manner will therefore also remain unknown.

No such difficulties are encountered in the following equations which relate  $r$  and  $R$  to the amount of ion which is complex-bound by protein.

By rearranging equation (3) we find for univalent cation

$$[CatPr] = \frac{r - R_{cat}}{r \times R_{cat}} [Cat]_b = \frac{r - R_{cat}}{r} [Cat]_f \quad (8)$$

And by rearranging equation (5) for univalent anion

$$[AnPr] = (R_{an} - r)[An]_b = \frac{R_{an} - r}{r} [An]_f \quad (9)$$



If we now consider the anions we find that according to our scheme the concentration ratio of the anions should give us the Donnan ratio – as Greene and Power assumed. But our scheme differs from reality since at physiological pH some Cl (about 1–3 %) is probably complex bound by serum albumin.

In the inside compartment the anion concentration found by chemical analysis should then represent  $[An^-]_i + [An\ Pr]$ . Consequently the concentration ratio  $R_{an}$  must be larger than the Donnan ratio since

$$R_{an} = \frac{[An]_i}{[An]_o} = \frac{[An^-]_i + [An\ Pr]}{[An^-]_o} > r \quad (4)$$

By following the same reasoning as in the case of the cations we can derive the following equation

$$r = \frac{[An]_o \times R_s - [An\ Pr]}{[An]_o} \quad (5)$$

Equations (3) and (5) can only give us  $r$  if for a given ion the concentration in the non protein solution, the concentration ratio and the amount that is complex bound in the protein solution are known.

If this is not the case then another approach is necessary. At the normal pH of serum  $[An\ Pr]$  will certainly be smaller than  $[Cat\ Pr]$ , and – provided  $[An\ Pr]$  is not more than 4 % of  $[An^-]_i$  and  $[Pr^-]$  can be determined accurately – it should be possible to calculate  $r$  with acceptable accuracy (i.e. to within 0.002) in the following manner

$$r = \frac{[Cat^+]_o}{[Cat^+]_i} = \frac{[An^-]_i}{[An^-]_o}$$

but also

$$\frac{[Cat^+]_o}{[Cat^+]_i} = \frac{[An^-]_o}{[An^-]_i + [Pr^-]} \quad (\text{see fig. 2})$$

therefore

$$r^2 = \frac{[An^-]_o}{[An^-]_i + [Pr^-]} \times \frac{[An^-]_i}{[An^-]_o}$$

and thus

$$r = \sqrt{\frac{[An^-]_i}{[An^-]_i + [Pr^-]}} \quad (6)$$

As  $An^-$  now figures in both numerator and denominator the effect of a systematic error due to inserting  $[An^-]_i$  instead of  $[An^-]_i$  will be minimised. When  $[An^-]_i$  is 4 % larger than  $[An^-]_i$  because of the presence of complex-bound anion the insertion of  $[An^-]_i$  in equation (6) will at normal plasma conditions, cause  $r$  to be calculated about 0.002 too high.

Equation (6) is similar to the corresponding equation given by VAN SLYKE *et al* (1923) and HASTINGS *et al* (1927). It differs in that it stresses the fact that it is concerned with dissociated ions only.

Equation (6) must be used with caution

When more than one type of anion is present in the solution the sum of the equivalent concentrations of all dissociated anions must be used ( $\Sigma[An^-]$ ). The error resulting from the practical necessity to use  $[An^-]_i$  instead of  $[An^-]_i$  has already been mentioned. Finally it should be remembered

ed that owing to the assumption of equal ionic strengths for plasma and for interstitial fluid no activity coefficients appear in the equation. In the second part of this chapter it has been argued that the cations balancing the negatively charged sites on the protein surface may have a lower activity coefficient than the other ions in the solution. If that case different activity coefficients would have to be inserted in equation (6) for  $[An^-]$  and for  $[Pr^-]$  in order to obtain the correct Donnan ratio. Neglect of the activity coefficients will lead to an erroneously low value for  $[Pr^-]$ . Conversely when  $r$  is known and equation (6) is used to calculate the negative net charge,  $[Pr^-]$ , the latter will be calculated too low. Although therefore in equation (6) the error due to the possible presence of  $AnPr$  is minimised, we have introduced with  $Pr^-$  new difficulty.

We have now three equations from which in theory  $r$  can be obtained: equation (3) or (5) can be used should it be known which fraction of cation or anion is complex bound by protein; equation (6) is available in case the concentration of filtrable anion in the protein-containing solution is known as well as protein net charge.

#### Calculation of quality and quantity of protein-bound ion

Since  $R_{cat}$  respectively  $R_{an}$  are related to  $r$  and to  $Cat/Pr$  respectively  $An/Pr$  (see equations (3) and (5)), the complex-bound fraction of an ion can be calculated when both its concentration ratio and the Donnan ratio are known. The equations differ somewhat according to whether the ion is univalent or bivalent.

We will first consider the univalent cation in fig. 2. By chemical analysis we find  $[Cat]_0$  and  $[Cat]_i$ . The difference between these two values to be indicated as  $\Delta Cat$  is related to the total amount of cation bound by protein. But whereas the latter is equal to  $[Cat/Pr] + [Pr^-]$ , it will be seen that  $\Delta Cat = [Cat]_i - [Cat]_0 = [Cat/Pr] + [Cat^+]_i - [Cat]_0$ , and  $\Delta Cat$  therefore underestimates the total amount of protein-bound cation. From fig. 2 it will be evident that the underestimate is equal to  $[An]_0 - [An]_i$  which according to equation (1) can also be written as  $(1 - r)[An]_0$ . And since  $[An]_0$  must be equal to  $[Cat^+]_0$  we find that

$$\text{protein-bound univalent cation} = \Delta Cat + (1 - r)[Cat]_0 \quad (7)$$

Provided  $r$  is known we should therefore be able to calculate the total amount of cation which is protein-bound. However as we discussed already the amount thus calculated is a minimum value since  $Pr^-$  stands in fact for the negative net charge. The total negative charge of the proteins may be considerably larger but the difference escapes detection since it is compensated by the positive charge of the proteins which we have no way of measuring. The amount of anion which is protein-bound in a salt-type manner will therefore also remain unknown.

No such difficulties are encountered in the following equations which relate  $r$  and  $R$  to the amount of ion which is complex-bound by protein.

By rearranging equation (3) we find for univalent cation

$$[Cat/Pr] = \frac{r - R_{cat}}{r \times R_{cat}} [Cat]_0 = \frac{r - R_{cat}}{r} [Cat]_i \quad (8)$$

And by rearranging equation (5) for univalent anion

$$[An/Pr] = (R_{an} - r)[An]_0 = \frac{R_{an} - r}{r} [An]_i \quad (9)$$

For bivalent cations the following equations should hold

$$\text{protein bound bivalent cation} = \Delta \text{Cat} + (1 - r^2) [\text{Cat}]_0 \quad (10)$$

and

$$[\text{Cat Pr}] = \frac{r^2 - R_{\text{cat}}}{r^2 \times R_{\text{cat}}} [\text{Cat}]_0 = \frac{r^2 - R_{\text{cat}}}{r^2} [\text{Cat}]_i \quad (11)$$

#### SOME FACTORS INFLUENCING THE DONNAN RATIO

The influence of protein concentration and the concentration of filtrable ions on the Donnan ratio

Equation (6) shows quite clearly that the magnitude of  $r$  depends on the relative concentrations of filtrable ions and protein. An increase in electrolyte concentration while protein concentration remains constant will increase  $r$ ; an increase in protein concentration while electrolyte concentration remains constant will decrease  $r$ . Fig. 3 shows

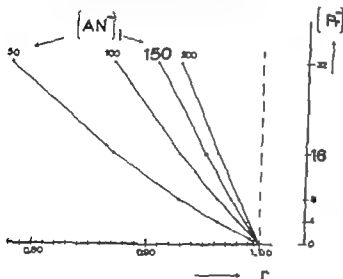


Fig. 3 Theoretical correlation between and negative net charge for different concentrations of An

the theoretical relationship between  $r$  and protein concentration at four different concentrations of filtrable ion. It can be seen that at the anion concentration to be expected in human serum the relation between protein concentration and  $r$  is nearly linear. Fig. 3 may serve to emphasize the need for details concerning protein and electrolyte concentration, when publishing values for the Donnan ratio. Furthermore, since the net charge of the proteins changes with pH, the latter must also be mentioned.

#### The presence of protein on both sides of the membrane

In our discussion of the Donnan equilibrium we restricted ourselves on purpose to the conditions existing if a protein solution such as serum is dialysed *in vitro* against an electrolyte solution containing no protein. This was the situation in our *in vitro* experiments (to be described later) and to them the equations given in the previous paragraph should be applicable without modification. In reality however the interstitial fluid contains protein, both mobile and fixed (connective tissue). The situation at the capillary

membrane is therefore different from that depicted in fig. 2 since charged macromolecules are present on both sides of the membrane. The effect on the Donnan ratio will be to increase it, since the latter is determined by the difference between the non-filtrable charges present on both sides of the membrane. If the charges balance each other it will become 1.000. At the capillary membrane we meet with the difficulty that the magnitude of the non-filtrable charges on the side of the interstitial fluid is unknown (chapter IV). Although therefore an accurate prediction is impossible, the Donnan ratio existing at the capillary membrane must be nearer 1.000 than the Donnan ratio found in *in vitro* experiments, where plasma or serum are equilibrated against protein-free solutions.

## REVIEW OF DATA CONCERNING THE INTERACTION BETWEEN ALBUMIN AND INORGANIC IONS

Albumin is the one plasma protein which has been obtained in sufficiently large quantities and in a sufficiently pure state to permit extensive *in vitro* studies to be made of its

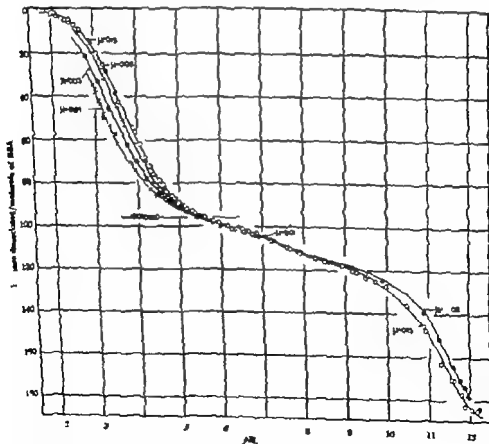


Fig. 4. H-ion titration curves of bovine serum albumin (at 25°C and at low ionic strengths). Reproduction of Fig. 1 in publication of T. A. Jones et al. (1935) Ann. N.Y. Acad. Sci., 77: 6414.

interaction with filtrable ions. Some of the results are of interest not only as an illustration of the interaction between filtrable ions and protein molecules in general but also because they will aid us in the interpretation of the results of our experiments with plasma and serum.

## INTERACTION BETWEEN ALBUMIN AND $H^+$ -IONS

**Titration curve** - The effects of temperature, ionic strength and the presence of other cations

Fig. 4 shows the titration curve obtained for a crystalline preparation of bovine serum albumin (TANFORD *et al* 1955). It will be seen that the total number of  $H^+$ -ions which can be dissociated per molecule of albumin is of the order of 200. Above pH 5.5 which is approximately the isoelectric pH the net charge is negative. The value of the latter increases from about 8 to about 16 when the pH increases from 6.9 to 7.8. This demonstrates that albumin is but a poor buffer substance in the physiological pH range. Changes in ionic strength appear to have no influence on this part of the curve. Changes in temperature have some effect as is shown in fig. 5. It was taken from an earlier publication of TANFORD (1950) and represents results obtained with a somewhat different

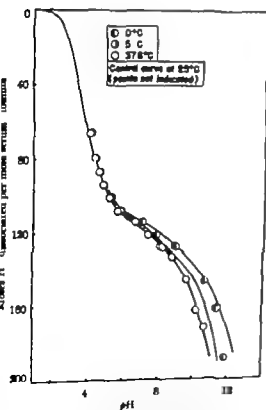


Fig. 5 -  $H^+$  ion titration curves of human serum albumin (mercapalbumin) at different temperatures (ionic strength 0.15). Reproduction of fig. 3 in publication of TANFORD (1950) *Ann. J. chem. Soc.* 72: 441

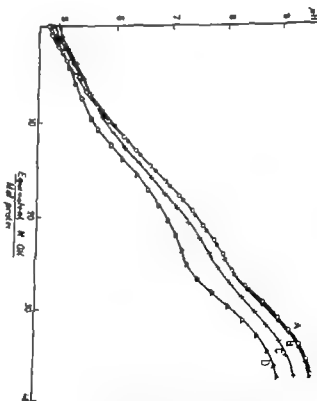


Fig. 6  $H^+$  ion titration curves of bovine serum albumin (at 25°C and at four different electrolyte concentrations). Reproduction of the figure in the publication of HARRIS (1954) *C. R. Acad. Sc. (Paris)* 239: 117. The original figure has been rotated ninety degrees clockwise for reasons explained in the text.

preparation of human serum albumin. The influence of the presence of other cations on the titration curve is demonstrated in fig. 6. It was taken from a publication by KEMPS (1954) and has been turned 90 degrees in clockwise direction in order to make ordinate and abscis comparable with those of the curves in fig. 4 and 5. Figure 6 then shows four titration curves of one non-crystalline preparation of bovine serum albumin.

Curve A was obtained when no salt was added to the albumin solution, curve B when the ionic strength was very low (between 0.001 and 0.003, by adding very small amounts of KCl), curve C when sufficient KCl was added to make the ionic strength 0.029, and curve D when sufficient  $\text{CaCl}_2$  was added to bring the ionic strength also to a value of 0.029. In each case the protein concentration was 4 g/l.

From fig. 6 it is evident that in the physiological pH range the type of ion present in the solution has a definite effect on the slope of the titration curve of albumin. Fig. 6 also shows that with decreasing  $\text{H}^+$ -ion concentration the other cations can compete more effectively with the  $\text{H}^+$ -ions for the dissociated groups on the protein molecules. This is especially so for the bivalent cations as is demonstrated by the fact that, when  $\text{CaCl}_2$  was added instead of KCl the number of  $\text{H}^+$ -ions dissociated per mole of albumin at pH 7.4 increased by about 5.

#### INTERACTION BETWEEN ALBUMIN AND CATIONS OTHER THAN $\text{H}^+$ -IONS

##### Competition between $\text{H}^+$ -ions and other cations

When fully dissociated an albumin molecule has some 200 binding sites for  $\text{H}^+$ -ions. They are not of equal strength and by ingenious analyses of titration data, TANFORD *et al.* (1955) distinguished the following ionogenic groups as binding sites for  $\text{H}^+$ -ions (in order of increasing strength of association the numbers between brackets indicate groups per mole of albumin):  $\beta$ - $\gamma$ -carboxyl (99), imidazole (16),  $\alpha$ -amino (1),  $\epsilon$ -amino (57), phenolic (19), guanidine (22) and sulfhydryl (1). In principle these sites should be available to all cations, but the  $\text{H}^+$ -ion has such advantages that in general the other cations can only compete if their concentration is considerably higher than that of the  $\text{H}^+$ -ions. This is reflected by the fact that the binding of other cations increases when the  $\text{H}^+$ -ion concentration decreases. The metal ions best suited to compete with the  $\text{H}^+$ -ions are constructive examples of this competition are given by TANFORD (1952) - are those of the intermediate series in the Periodic System ( $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Co}^{2+}$  a.o.). This high affinity is explained by a typical electron structure, but probably because of this very characteristic the number of binding sites for which these ions can compete effectively with  $\text{H}^+$ -ions is limited. For details the reader may be referred to GURD and WILCOX' lucid and instructive review (1956). An expression of the specialized character of this complex type of cation binding is the fact that it can occur at a pH value where the net charge of the protein is zero or even positive.

For the metal ions mentioned the tendency to become attached by complex binding is so strong that their interaction with proteins can be considered to be solely of this type. This is probably not so for the alkaline earth metal ions and certainly not so for the univalent alkali metal ions.

### Interaction with $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$

These bivalent cations are partially complex bound presumably at ionogenic groups which provide an opportunity for chelation. There is however evidence that in the physiological pH range some of the  $\text{Ca}^{2+}$  ions are not bound in true complex-compounds, but attracted by electrostatic forces of sufficient strength to decrease the activity coefficient of these  $\text{Ca}^{2+}$  ions below the value calculated from the ionic strength of the solution (see for instance GURD & WILCOX 1956). For reasons discussed already association constants calculated for  $\text{Ca}^{2+}$  binding by proteins have therefore a somewhat dubious value.

### Interaction with $\text{Na}^+$ and $\text{K}^+$

These univalent cations are apparently bound by albumin in a salt type manner only up to pH values as high as 8.0 (SCATCHARD *et al.* 1950<sup>1</sup>, CARR 1956, VAN OS & KOOPMAN-VAN EUPEN 1957). But neither Scatchard's experiments nor those of the Dutch workers can exclude the possibility that at physiological pH values up to 5/ of the  $\text{Na}^+$  and  $\text{K}^+$  present in solution are bound in a complex type of manner.

The experiments of Scatchard *et al.* were done at a pH value near the IEP and they involved subtraction of results obtained by different methods at different temperatures. Van Os and Koopman-Van Eupen did their experiments at a pH of about 7.70 but in some of the experiments the conductance of Na- and K-albuminate was found to be 5-10% lower than that of NaCl and KJ.

CARR used a special technique with measurement of membrane potentials and in his experiments the margin of uncertainty appears to be smaller. However the concentration of cation was rather low (5-20 mM/L) in comparison to the protein concentration (about 30 g/L). Other experiments suggest that the amount of complex bound ion depends on the concentration of the particular ion (see for instance for  $\text{Ca}^{2+}$  CARR 1953<sup>1</sup>) and Carr's results do not therefore exclude the possibility that at physiological concentrations a small amount of  $\text{Na}^+$  (1-2%) is bound by albumin in a complex type of manner. It should moreover be mentioned that Carr found a definite decrease in  $\text{Na}^+$  and  $\text{K}^+$  activity in the presence of (non purified)  $\alpha$ - and  $\beta$ -globulins and fibrinogen.

The results given in Table IV of Carr's publication (1956) indicate that at pH 7.5 and in the presence of about 5-20 mEq/L cation, 2-3 mEq of  $\text{Na}^+$  are complex-bound per 100 g of  $\alpha$ - and  $\beta$ -globulins. Expressed as a percentage of total cation concentration, complex-bound  $\text{Na}^+$  was of the order of 10-20%.

Of particular interest are the experiments of DOREMUS & JOHNSON (1958), who used measurements of conductance and transference to study the interaction of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  with (reasonably purified) bovine serum albumin. They considered that a small ion is bound to a protein ion when the former migrates with the protein ion under the conditions of the electrolysis experiment. This experimental approach is apt to give a fairly good indication of the total amount of ion attracted both by electrostatic forces and by complex binding. Their results indicate that  $\text{Na}^+$  is without doubt bound by albumin (10 ions per molecule at a net negative charge of about 25), but the authors concluded that the sodium ions form only weak ion pairs with the protein ion charges or even that the small ions are merely held in the charged sphere of the whole protein ion. This situation is best explained by pure electrostatic interaction and in the summary they state that the appreciable sodium binding found was primarily electrostatic.

The fact that the results of Dorosius and Johnson are sometimes quoted as evidence for complex binding of Na by albumin is an example of the misunderstandings which arise from the use of the term "binding" in more than one sense.

We can conclude that the experimental evidence suggests that albumin binds the alkali metal ions in a salt-type manner only but that it cannot exclude the possibility that up to 2% of the Na and K present in an albumin solution is bound in a complex-type manner.

## INTERACTION BETWEEN ALBUMIN AND ANIONS

Albumin is rather remarkable in its affinity for inorganic and organic anions at pH values well above its IEP as GROLLMAN (1925) was the first to demonstrate.

### Interaction with Cl

In the context of this study the most interesting interaction is that with Cl<sup>-</sup> which was detected by SCATCHARD *et al.* in consequence of the unexpected deviation in osmotic pressure of an albumin solution in 0.15 molar NaCl solution (SCATCHARD *et al.*, 1944). Subsequent studies revealed the fact that at pH values near the IEP Cl<sup>-</sup> activity decreased considerably when albumin was added (SCATCHARD *et al.*, 1950<sup>1</sup>) and thiocyanate activity even more so (SCATCHARD *et al.*, 1950<sup>2</sup>). We have been unable however to find in the articles published by SCATCHARD *et al.* data on Cl<sup>-</sup> binding at physiological pH with two exceptions.

Firstly in their original publication in the *Journal of Chemical Investigation* (SCATCHARD *et al.*, 1944) and in two following papers (SCATCHARD 1949 and SCATCHARD *et al.* 1946) the deviation from the expected osmotic behaviour subsequently proved to be due to complex-type binding of Cl<sup>-</sup> appears to be very slight at pH 7.00 and insignificant at pH 7.40.

Secondly SCATCHARD *et al.* (1937), reporting experiments with exchange-electrodes, give in Table IV of their publication the following three values for moles Cl<sup>-</sup> bound per mole albumin at pH values above 5.5: 1.49 at pH 7.24, 0.27 at pH 8.08 and -2.71 at pH 10.31. The values refer to solutions containing  $\pm 10$  g/L bovine serum mercapt-albumin and  $\pm 10$  mM/L of NaCl and NaOH each. With increasing concentration of NaCl but constant protein concentration the amount of protein-bound Cl<sup>-</sup> increases. From experiments with serum albumin and NaCl at pH  $\pm 7.0$  (SCATCHARD *et al.*, 1940<sup>1</sup>) it would appear that an increase in Cl<sup>-</sup> concentration from  $\pm 7$  to 140 mEq/L at an albumin concentration of 70 g/L doubles the amount of protein-bound Cl<sup>-</sup>. Assuming a similar factor to apply in the higher pH range, SCATCHARD's observation at pH 7.34 might be interpreted to indicate Cl<sup>-</sup> binding of about 3 moles per mole albumin at pH  $\pm 7.20$ . This would be equal to about 2 mEq of Cl<sup>-</sup> per 40 g albumin, the latter being the amount present in one litre of normal serum.

Other authors have extended their measurements to include a pH value of 7.0. ALBERTY & MARIN (1951) using bovine serum albumin found (at pH 7.0, temp. 0°C, albumin concentration 8 g/L and NaCl 0.13 mole/L) the rather high value of 7.6 mole Cl<sup>-</sup>/mole albumin. CARR (1953<sup>2</sup>) studied a number of proteins at pH values ranging from 3.0 to 7.0. His data are well suited for estimating the amount of protein-bound Cl<sup>-</sup> at physiological pH.

In the first place his results indicate that at pH higher than 5.5 serum globulins bind no Cl<sup>-</sup> in complex-type manner. But for our present discussion the most important data are to be found in his graphical presentation (fig. 2 in the 1953<sup>2</sup> publication) of moles of Cl<sup>-</sup> bound per 10<sup>4</sup> g protein over the pH range 3.0 to 7.0. From this graph it would appear that at Cl<sup>-</sup> concentration



of 100-120 mEq/L the following amounts are bound by serum albumin (moles  $\text{Cl}^-$  per  $10^4$  g protein). At pH 5.0 10.6, at pH 5.5 6.9 at pH 6.0 4.2, at pH 6.5 2.6 and at pH 7.0 1.7 By extrapolation we find a value of 1.4 at pH 7.4. At this pH then 40 g albumin would bind about 0.6 mEq  $\text{Cl}^-$ .

The evidence suggests that some  $\text{Cl}^-$  is complex bound by albumin at physiological pH but the amount is somewhat dubious. Scatchard's own data and those of Carr indicate that at pH 7.40 a value between 0.5 and 3.0 mole  $\text{Cl}^-$  per mole albumin is the most reasonable estimate. It must be stressed that this refers to purified preparations of albumin in solutions containing no bivalent cations.

#### Interaction with $\text{HCO}_3^-$

Concerning the interaction between  $\text{HCO}_3^-$  and albumin very little is known. KLOTZ & URQUHART (1949) observed that albumin shows a slight decrease in its affinity for methylorange in the presence of bicarbonate ions (concentration of the latter 100 mEq/L). Presumably  $\text{HCO}_3^-$  competes with methylorange for binding sites on the albumin molecules. In the experiments of Klotz and Urquhart the pH was 8.60 and it is conceivable that the observed effect would have been more pronounced at pH values around 7.40. But quantitative data on  $\text{HCO}_3^-$ -binding are not available.

### THE MEASUREMENT IN PLASMA (OR SERUM) OF PROTEIN BOUND ION NET PROTEIN BOUND CATION (NCE) AND OF PROTEIN NET CHARGE

#### THE IMPORTANCE OF STUDYING PLASMA OR SERUM

When we aim at understanding phenomena such as the ion equilibrium at the capillary wall and the NCE and net charge of the plasma proteins it is necessary to study ion protein interaction in plasma or at least in serum. Extrapolation from results obtained with purified protein fractions to the conditions existing in plasma is not without danger in the case of ions such as  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  which are to a very large or a large extent bound in a salt type manner. In the case of the ions that are almost exclusively complex bound ( $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$  etc.) the results of experiments with purified proteins are in general applicable to plasma because true complex binding is relatively insensitive to changes in pH, in ionic strength and in the nature of the other ions present in solution. But the salt type of binding is much more sensitive and in its case conclusions reached from observations on relatively simple solutions are not necessarily valid for plasma.

#### ASSUMPTIONS MADE WHEN STUDYING ION PROTEIN BINDING IN PLASMA OR SERUM

In order to interpret ion ion interaction in a simple solution of strong electrolytes a number of simplifying assumptions has to be made. Their number increases when ion binding by purified proteins is studied and when ion protein binding is analysed in solutions such as plasma the simplifications involved become considerable. The most important which we will have to make are

(1) In as far as NCE and protein net charge are concerned plasma is essentially an aqueous solution containing  $H^+$   $Na^+$   $K^+$   $Ca^{2+}$   $Mg^{2+}$   $Cl^-$   $HCO_3^-$  and protein

The contribution of other cations present in plasma is negligible under *in vivo* conditions, but this is not so for the other anions (chapter II, IV and V).

(2) Plasma has the same ionic strength as interstitial fluid.

The consequences of this assumption have already been discussed.

(3) Protein is the only non-filtrable substance in plasma.

(4) The plasma proteins can be treated as if they constitute one protein.

The last two assumptions determine the meaning of the term protein as used in our studies of plasma or serum. In order to be able to discuss this meaning we will first briefly describe the manner in which we have determined ion-protein binding and in what way the values obtained are expressed.

#### DETERMINATION OF PROTEIN-BOUND ION IN THE EXPERIMENTS TO BE REPORTED AND EXPRESSION OF THE VALUES OBTAINED

Essentially we have determined ion-protein binding by comparing solutions of equal ionic composition but with different concentrations of protein. Observed differences between the concentrations of the filtrable ions were correlated with the concomitant differences between the concentrations of protein. The concentration of the filtrable ions is expressed in mEq per Kg  $H_2O$  that of protein in g per Kg  $H_2O$ . Protein-bound ion can therefore be calculated in mEq per Kg solvent water or in mEq per unit weight of protein. For the latter we have chosen 100 g, since plasma protein cannot be expressed in moles. In the same way the difference between the sum concentration of the filtrable cations and the sum concentration of the filtrable anions in a sample can be correlated to the protein concentration in order to obtain NCE. The latter is then again calculated either in mEq per Kg solvent water or in mEq per 100 g of protein.

Samples in which the protein concentration was presumably the only independent variable were obtained by means of the two methods already mentioned in the General Introduction, i.e. *in vivo* ultrafiltration (chapter IV) and equilibrium dialysis *in vitro* (chapter V).

#### THE MEANING OF THE TERM 'PROTEIN' IN THE STUDIES TO BE REPORTED

With both methods one actually observes the effect not of protein per se but of all non-filtrable substances present in plasma. Since the observed effect is correlated with the total protein concentration as determined by the biuret method we do in fact correlate the amount of filtrable ion bound by all non-filtrable plasma substances with the amount of plasma protein. Since the latter is not necessarily the only non-filtrable substance present in plasma (which also contains for instance polysaccharides) an unknown error is introduced. In normal plasma this error is presumably negligible, but in pathological plasma it may be significant. This must be kept in mind when the results obtained with plasma are compared with those obtained with purified protein fractions.

Furthermore the proteins in plasma are by no means pure. They have associated with varying amounts of inorganic ions, with saccharides, with steroids, with fatty acids etc. Indeed the biological importance of the plasma proteins is to a large extent determined by their ability to bind substances in a reversible manner. The latter characteristic was stressed years ago by BENNHOLD (1932) and described as *Vehiclefunktion*. More recently KLOTZ and co-workers have demonstrated that this tendency exists towards numerous inorganic and organic ions. They remark (KLOTZ & URQUHART 1949) that the ion protein complexes thus formed may differ appreciably in physicochemical properties from those to be anticipated for the simple protein molecule (for which we can read the purified protein fraction). It is therefore quite possible that the same plasma protein will be found to behave differently towards the inorganic ions, according to whether normal plasma is studied or for instance, uraemic or hyperlipaemic plasma.

Finally when for any given plasma the concentration of the filtrable ions is correlated with the total protein concentration it is tacitly assumed that the relative contribution of the various plasma proteins to the total protein concentration is the same in the samples that are compared. In chapter IV the correctness of this assumption in the case of the methods used will be tested. When results obtained for different plasmas are compared it must be realised that a difference in the amount of ion bound per 100 g of protein may be due to either a difference in the composition of (total) protein or to an abnormally low or high ion binding by one or more of the plasma proteins. Judicious comparison of the results obtained for plasma samples with different protein composition may then provide an answer.

#### THE DETERMINATION OF PROTEIN NET CHARGE IN PLASMA

For the purpose of this study net charge was defined as the difference between the equivalent concentrations of dissociated filtrable cations and dissociated filtrable anions. According to this definition the magnitude of net charge is equal to the difference between the amounts of cations and of anions that are bound in a salt-type manner and it is expressed in mEq per Kg solvent water or mEq per 100 g of protein. This indirect definition is necessary because, in contrast to solutions containing one well defined protein, one cannot in the case of plasma calculate net charge from the titration curve of the protein and the dissociation constants of its acid groups. Moreover the number of purified proteins of which the titration curve is accurately known is very small. Among these is serum albumin but it remains to be seen whether the titration curve is the same for albumin such as it is in plasma. It is impossible to obtain a complete titration curve for plasma because of the instability of many globulins. Titration of plasma over the physiological pH range gives information concerning the overall buffer capacity for that pH range but the presence of weak acids and bases other than protein prevents the interpretation of such titration data in terms of protein net charge.

Although we cannot determine the concentrations of dissociated cations and anions in plasma the above mentioned definition of protein net charge has the advantage of relating it to NCE and to the amounts of complex bound cation and anion (see page 17). Since NCE can be determined and presumably the amounts of complex bound cation and anion also (see page 23) a value should be obtainable for net charge. But

It has already been pointed out that this value is not easily interpreted in terms of a definite number of charged groups on the protein molecules.

# SOME OF THE RELATIONS POSSIBLE BETWEEN THE NCE AND THE NEGATIVE NET CHARGE OF THE PROTEINS IN PLASMA

Fig. 7 is an elaborate version of fig. 2 and it shows some of the relations possible in the physiological pH range. Four different types of protein molecules are compared. They

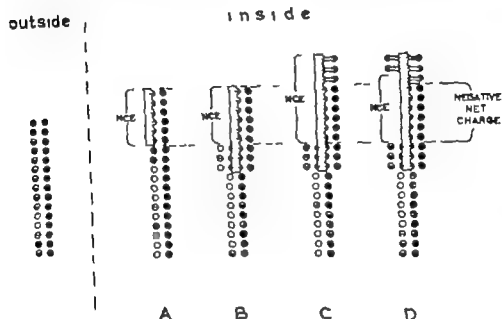


Fig. 7 Schematic representation of possible relationships between NCE and negative net charge of the proteins in plasma (physiological pH range).

all have the same negative net charge, but they differ in regard to the concomitant presence of a positive charge on the protein and in regard to their tendency to bind filtrable cations and anions in a complex-type manner. All four protein solutions are considered to be in equilibrium through a semipermeable membrane with the same electrolyte solution. Since the four proteins carry the same negative net charge and since the concentration of filtrable dissociated anions in the inside solution is the same in every case, the Donnan ratio is also the same for each of the four equilibria.

*Equilibrium A* represents the most simple possibility. The protein molecule carries a negative charge only and there is no complex-type of binding. NCE is therefore equal to the total negative charge on the protein, which in this case is also the net charge. Furthermore, the concentration ratios of the cations and the anions are identical with the Donnan ratio.

*Equilibrium B* represents a protein molecule which carries both negative and positive charges but more negative ones. There is no complex type of binding. NCE is again

equal to the negative net charge, but the latter is smaller than the total negative charge. The concentration ratios of cations and anions are still identical with the Donnan ratio.

*Equilibrium C* The protein carries now both negative and positive charges but in addition some cation is complex-bound. NCE exceeds therefore the negative net charge by an amount equal to the equivalents of cation that are bound in a complex type manner. Whereas the concentration ratio of the anions is still identical with the Donnan ratio the concentration ratio of the cations is smaller.

*Equilibrium D* In addition to carrying both negative and positive charges and binding some cation in a complex type of manner the protein now also binds some anion in this manner (in our example less anion than cation). In this case NCE is again larger than the negative net charge but the difference is less than in the case of C since the complex bound anion partially balances the complex bound cation. For both the cations and the anions the concentration ratio now differs from the Donnan ratio but whereas the concentration ratio will be smaller in the case of the cations it will be larger in the case of the anions.

According to the data obtained with purified protein fractions it would appear that the situation described under D is the most likely one to be encountered in normal plasma.



### *Summary of chapter I*

For the purpose of the present study plasma is considered an aqueous solution containing filtrable inorganic ions ( $H^+$ ,  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Cl^-$ ,  $HCO_3^-$ ) and non filtrable organic ions (protein).

The various types of interaction between the inorganic ions and the proteins are discussed, and binding is defined as attraction of ions by protein regardless of the nature and the strength of the attracting forces. For practical purposes binding is distinguished in two types according to its effect on the activity coefficient of the ions. When the activity coefficient is not different from that found in a solution of equal ionic strength and composition but containing no protein, the binding is considered to be of the salt type, i.e. the protein-bound ion behaves as if fully dissociated. When however the activity coefficient decreases in the presence of protein the binding is considered to be of the complex-type i.e. protein bound ion behaves as if it is no longer dissociated. The difference between the two types of binding becomes manifest when solutions such as plasma are subjected to equilibrium dialysis. As the Donnan equilibrium concerns active ions only the presence of ion bound in a complex type manner will cause the concentration ratio of the particular ion to differ from the Donnan ratio.

Plasma contains many different ions and proteins, the latter carrying both positive and negative charges. A measure of the overall effect of ion protein binding is the difference between the sum concentration of the filtrable cations and the sum concen

tration of the filtrable anions. This net cation equivalency or NCE (old term 'base binding power') is an empirical quantity and should not be mistaken for the negative net charge of the proteins. The latter is equal to the difference between the amounts of cation and anion bound in a salt type manner whereas NCE is equal to the difference between the total amounts of protein-bound cation and anion and includes therefore the ions bound in complex-type manner.

The pragmatic nature of the definitions given is stressed.

*Review of literature concerning the net cation equivalency of plasma proteins; the normal concentration of the main filtrable ions in plasma and the distribution of the latter between plasma and interstitial fluid*

This review is mainly devoted to data concerning NCE of the plasma proteins and data concerning the interaction between the latter and the univalent ions. For recent reviews concerning the interaction between Ca and the plasma proteins the reader is referred to TORIBARA *et al* (1957) to TEREPA *et al* (1958) to PRASAD (1960) and to WALSER (1961 1962). For Mg we refer to HASSELMAN & VAN KAMPEN (1958) to PRASAD (1961) and to WALSER (1961).

Leaving out the alkaline earth metal ions considerably reduces the amount of literature to be reviewed. But even so it is impossible to be complete, especially where the older literature is concerned. Only those publications will be cited which provided new data, presented a new method or contributed in any other way materially to the elucidation of the problem under discussion. The literature from the previous century often makes fascinating reading because of the thoughts which were already developed then and the often painstaking documentation of the experimental results. As an example we should like to mention the work of SCHMIDT (1850) who was apparently the first to determine electrolyte concentrations in human serum and the work of LOEWY & ZUNTZ (1894) who as far as we know made the first attempt to determine die Bindung der Alkalien in Serum. From dialysis experiments with horse serum they concluded that *ein Theil des Alkalis derart gebunden ist dass es am Diffusionsprozesse nicht theilnehmen kann*.

### NCE OF PLASMA PROTEINS (NORMAL PLASMA)

According to its definition NCE is obtained by subtracting the sum concentration of the filtrable anions from the sum concentration of the filtrable cations. However the accurate determination of both these concentrations is no easy matter. In fact only Van Slyke and co-workers Darrow & Hartmann and Henderson *et al* have attempted to determine the value of NCE accurately. To this end they first removed all filtrable electrolytes from the plasma proteins and then redissolved the latter in a solution containing only Na, Cl and  $\text{HCO}_3$  ions. A few authors have subsequently tried to verify the values found by the Van Slyke group by comparing these values with those obtained when for plasma or serum the sum of the concentrations of Cl,  $\text{HCO}_3$ , inorganic phosphate and an assumed value for ( $\text{SO}_4$  + organic acids) was subtracted from the sum concentration of Na, K, Ca and Mg.

We will first present and discuss the work of VAN SLYKE and associates and that of DARROW & HARTMANN and of HENDERSON *et al*. Later we will make an estimate of

NCE from the values reported for the normal plasma concentrations of Na, K, Ca, Mg, Cl,  $\text{HCO}_3$  and inorganic phosphate

# THE EXPERIMENTS OF VAN SLYKE AND CO WORKERS AND ADDITIONAL EXPERIMENTS DONE BY DARROW & HARTMANN AND BY HENDERSON AND CO WORKERS

As the value found by Van Slyke and co-workers is the one always quoted their experimental evidence must be discussed in some detail. They determined NCE in the following manner. To a protein solution which had been freed from all electrolytes other than H-ions by prolonged dialysis against distilled water an accurately known amount of  $\text{NaHCO}_3$  and  $\text{NaCl}$  (Na and Cl in physiological concentration) was added and the resulting solution equilibrated at  $38^\circ\text{C}$  with  $\text{CO}_2$  at various pressures.

Water content was determined by drying at  $110^\circ\text{C}$  to constant weight, protein concentration by macro-Kjeldahl analysis, accepting a nitrogen factor of 6.25 total  $\text{CO}_2$  by the manometric method. Procs by Haldane analysis of the gas

$$\text{H}_2\text{CO}_3 \text{ from } \frac{1000 \cos}{22.26} \frac{P_{\text{CO}_2}}{760} \text{ where } \cos$$

had been determined in previous experiments.  $\text{HCO}_3$  from total  $[\text{CO}_2]$   $[\text{H}_2\text{CO}_3]$  pH in most experiments by means of the hydrogen electrode at  $38^\circ\text{C}$ . Na and Cl concentration were calculated from the weighed amount of  $\text{NaHCO}_3$  and  $\text{NaCl}$  added to the protein solution. In some cases as control total base was determined by the method of Fluke

As the solutions were considered to contain only one type of cation (Na<sup>+</sup>), and three types of anion ( $\text{Pr}^-$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$ ) it was concluded that  $[\text{Na}^+] = [\text{Pr}^-] + [\text{HCO}_3^-] - [\text{Cl}^-]$  (H<sup>+</sup> and OH<sup>-</sup> can be neglected for quantitative calculations). The possibility of the proteins attracting anions was therefore not considered and ion-protein binding was tacitly assumed to be of the salt-type only. By subtracting the sum concentration of  $\text{Cl}$  and  $\text{HCO}_3$  from the Na concentration of the solutions studied Van Slyke *et al* obtained the amount of 'base bound' by protein. In their equations this quantity was indicated by BP and from chapter I it will be evident that it is identical with NCE. From the protein content of the solution BP could then also be calculated in terms of mEq per unit weight of nitrogen or protein. By determining BP at various values of pH, a relation between BP and pH was obtained. Thus Van Slyke and co-workers expressed as d BP BP dpH (that is to say increase in BP per unit increase in pH) and termed molecular buffer value

The method was first applied in the determination of the NCE of crystalline Hb and  $\text{HbO}_2$  (VAN SLYKE *et al* 1922, HASTINGS *et al* 1924). In their classic paper on the factors controlling the electrolyte and water distribution in blood, VAN SLYKE, WU & McLEAN (1923) report for the first time experiments on the base binding power of serum proteins. Two samples of horse serum were used, which had been dialysed until less than 0.001 N electrolyte per litre remained. The protein concentration of the samples was 50.3 g/L and 47.2 g/L respectively the Na concentration 130.5 mEq/L. For each sample BP was determined at four pH values, varying from about 7.10 to 7.74. As a linear relation was found between pH and BP the following equation was calculated



*Review of literature concerning the net cation equivalency of plasma proteins the normal concentration of the main filtrable ions in plasma and the distribution of the latter between plasma and interstitial fluid*

This review is mainly devoted to data concerning NCE of the plasma proteins and data concerning the interaction between the latter and the univalent ions. For recent reviews concerning the interaction between Ca and the plasma proteins the reader is referred to TORIBARA *et al* (1957) to TEREPKA *et al* (1958) to PRASAD (1960) and to WALSER (1961, 1962). For Mg we refer to HASSELMAN & VAN KAMPEN (1958), to PRASAD (1961) and to WALSER (1961).

Leaving out the alkaline earth metal ions considerably reduces the amount of literature to be reviewed. But even so it is impossible to be complete, especially where the older literature is concerned. Only those publications will be cited which provided new data, presented a new method or contributed in any other way materially to the elucidation of the problem under discussion. The literature from the previous century often makes fascinating reading because of the thoughts which were already developed then and the often painstaking documentation of the experimental results. As an example we should like to mention the work of SCHLUDT (1850) who was apparently the first to determine electrolyte concentrations in human serum and the work of LOEWY & ZUNTZ (1894) who as far as we know made the first attempt to determine die Bindung der Alkalien in Serum. From dialysis experiments with horse serum they concluded that ein Theil des Alkalis derart gebunden ist dass es am Diffusionsprozesse nicht theilnehmen kann.

### NCE OF PLASMA PROTEINS (NORMAL PLASMA)

According to its definition NCE is obtained by subtracting the sum concentration of the filtrable anions from the sum concentration of the filtrable cations. However the accurate determination of both these concentrations is no easy matter. In fact only Van Slyke and co-workers Darrow & Hartmann and Henderson *et al* have attempted to determine the value of NCE accurately. To this end they first removed all filtrable electrolytes from the plasma proteins and then redissolved the latter in a solution containing only Na, Cl and  $\text{HCO}_3$  ions. A few authors have subsequently tried to verify the values found by the Van Slyke group by comparing these values with those obtained when for plasma or serum the sum of the concentrations of Cl,  $\text{HCO}_3$ , inorganic phosphate and an assumed value for ( $\text{SO}_4$  + organic acids) was subtracted from the sum concentration of Na, K, Ca and Mg.

We will first present and discuss the work of VAN SLYKE and associates and that of DARROW & HARTMANN and of HENDERSON *et al*. Later we will make an estimate of

In their publication the protein values were expressed in grams of N. To make the equation comparable with the older one it has been expressed in terms of grams of protein assuming an N factor of 6.25, as this was the factor which Van Slyke used for calculating equation (D).

In normal human serum Van Slyke *et al* found with their fractionation technique the ratio albumin-globulin to be 1.6. From (C) they calculated therefore for normal human serum the equation

$$BP = 0.104 P (\text{pH} - 5.08) \quad (D)$$

According to (D) the 'base binding power' that is the NCE, of 100 g human serum protein (at pH 7.35 and 38°C) is 23.6 mEq and increases 10.4 mEq per unit pH.

The correctness of extrapolating the values found with horse serum proteins to human serum was tested with one sample of pooled human serum which had been dialysed for three months at 0°C. It contained 17.2 g/Kg H<sub>2</sub>O albumin and 14.4 g/Kg H<sub>2</sub>O globulin. The CO<sub>2</sub> titration was carried out at 4 different values of pH (6.01 to 7.97), the Na concentration was 136 mEq/Kg H<sub>2</sub>O and the temperature 38°C. At every pH the BP found was 5-7% lower than the value calculated from equation (D). At the end of their article Van Slyke *et al* commented on the fact that the earlier equations indicated a considerably lower value for BP. They concluded that probably in the earlier experiments dialysis had not been pushed far enough to remove the last traces of pre-existing base from the protein preparations used.

Equation (C) and (D) have generally been accepted, and the value of 16 mEq/L inserted in the usual cation-anion diagrams for plasma or serum is based on them. Table 1 summarises the results obtained in the experiments of Van Slyke and co-workers. The NCE values were calculated for pH 7.35 and expressed in mEq per 100 g protein. This was done because in our experiments to be discussed later all values have been expressed per 100 g protein. The pH 7.35 was selected because generally not arterial but venous blood is analysed, which in the resting forearm appears to have a pH of on the average 7.35.

It should be stressed that Van Slyke *et al* did their experiments at 38°C. The observations of S. LOW *et al.* (1925) on the effect of temperature on acid-base-protein equilibrium in the pH range 7.00-7.70 suggest that whereas the microclastic buffer value of the plasma proteins is not affected when the temperature changes from 38°C to 20°C, the apparent IEP increases by 0.02 on the average for each centigrade decrease in temperature. Accordingly the value of NCE should be about 3.3 mEq/100 g protein lower at 20°C than at 38°C, other conditions remaining equal.

In view of the accuracy needed the experiments of Van Slyke are not easily repeated and it is therefore not surprising to find that only two other attempts have been made to determine the NCE of the plasma proteins.

In 1929 DARROW & HARTMANN pointed out that equation (B) of Van Slyke and co-workers could not be used to calculate 'base binding power' in patients in whom the A/G ratio was abnormal. They did therefore some experiments with beef serum which had been dialysed for 2 to 3 days against distilled water in an atmosphere of CO<sub>2</sub> and in the cold. During the dialysis part of the globulins precipitated.

After dialysis CO<sub>2</sub> titration after the manner of Van Slyke *et al.* was carried out separately on both serum, on the washed precipitated globulins and on the proteins not precipitated by dialysis. Total protein was determined by the macro-Kjeldahl method using a factor of 6.25. Albumin and globulin were determined in the same way after fractionation by 22.2 per cent disodium sulfate. Apparently two samples were studied, both at two pH values (about 7.2 and about 7.5).

(for the pH range covered by the experiments)

$$BP = 0.068 P_s (\text{pH} - 4.80) \quad (\text{A})$$

where BP represents base bound by protein (*i.e.* NCE) in mEq and  $P_s$  serum protein in grams (The pH at which BP would be zero according to the equation - in this case 4.80 - will be indicated as "apparent IEP") According to equation (A) the NCE of 100 g of normal horse protein is 17.4 mEq at pH 7.35 and increases by 6.8 mEq for every 1.0 unit increase in pH. It is interesting to note that the values used in the general equations from which the authors calculated the Donnan ratios across the erythrocyte membrane and across the capillary wall are based on this equation.

In 1927 in a paper concerned with the distribution of electrolytes between transudates and serum, HASTINGS *et al.* (1927) pointed out that equation (A) correlated BP with total protein only and that - as the titration curves of albumin and globulin differed greatly and moreover the albumin/globulin ratio was not the same in man as in the horse - the equation could not be used to calculate the base bound by human serum proteins. They mentioned experiments in which the method described above was applied to dialysed human serum. From these experiments the following relation was calculated for human serum:

$$BP = 0.097 P (\text{pH} - 5.26) \quad (\text{B})$$

Equation (B) indicates a NCE of 20.3 mEq per 100 g of (normally constituted) human serum at a pH of 7.35 increasing with 9.7 mEq for every 1.0 pH unit increase. The experimental data from which equation (B) was derived have never been published as far as we could ascertain.

Instead, another equation was proposed in 1928 by VAN SLYKE *et al.* It is based on the results obtained with albumin and globulin fractions of horse serum. The globulin was salted out from serum with half saturated  $(\text{NH}_4)_2\text{SO}_4$ , albumin with saturated  $(\text{NH}_4)_2\text{SO}_4$  and both fractions were freed from small electrolytes by pressure dialysis against distilled water which had been saturated with  $\text{CO}_2$  to bring its reaction near to the isoelectric point of the protein fractions. In this study the protein solutions were not only titrated with  $\text{CO}_2$ , but with HCl also. For that purpose the protein fractions were dissolved in enough NaOH to render the solution distinctly alkaline, and then titrated electrometrically with 0.1 N HCl. The amount of cation opposed by protein valences was found by subtracting the HCl added from the NaOH originally present in the solution.

In three experiments 2 preparations of albumin (protein concentration about 50 g/Kg  $\text{H}_2\text{O}$ ) were studied, and in another three experiments 2 preparations of globulin (protein concentration 70.9 and 36.2 g/Kg  $\text{H}_2\text{O}$ ). The Na concentration was between 135 and 139 mEq/Kg  $\text{H}_2\text{O}$  and all experiments were done at 38°C. Determinations were done at 3 to 7 different pH values, ranging from 5.20 to 9.26, but in the final analysis the authors have only included the values obtained at pH values between 6.50 and 7.80. In the calculation of  $\text{HCO}_3^-$  a value of 6.11 was assumed for pK of carbonic acid.

The relation between pH and BP was again a linear one for both protein fractions and no difference was found between the results obtained with  $\text{CO}_2$  and with HCl titration. From their results the following equation was calculated for horse serum:

$$BP = 0.124 \text{ Alb} (\text{pH} - 5.16) + 0.077 \text{ Glob} (\text{pH} - 4.89) \quad (\text{C})$$

In their publication the protein values were expressed in grams of N. To make the equation comparable with the older ones we have expressed it in terms of grams of protein assuming an N factor of 6.25, as this was the factor which Van Slyke used for calculating equation (D).

In normal human serum Van Slyke et al. found with their fractionation technique the ratio albumin-globulin to be 1.6. From (C) they calculated therefore for normal human serum the equation

$$BP = 0.104 P \quad (pH = 5.08) \quad (D)$$

According to (D) the "base binding power" that is the NCE, of 100 g human serum protein (at pH 7.35 and 38°C) is 23.6 mEq and increases 10.4 mEq per unit pH.

The correctness of extrapolating the values found with horse serum proteins to human serum was tested with one sample of pooled human serum which had been dialysed for three months at 0°C. It contained 17.2 g/Kg H<sub>2</sub>O albumin and 14.4 g/Kg H<sub>2</sub>O globulin. The CO<sub>2</sub> titration was carried out at 4 different values of pH (6.01 to 7.97), the Na concentration was 136 mEq/Kg H<sub>2</sub>O and the temperature 38°C. At every pH the BP found was 5-7% lower than the value calculated from equation (D). At the end of their article Van Slyke et al. commented on the fact that the earlier equations indicated a considerably lower value for BP. They concluded that probably in the earlier experiments dialysis had not been pushed far enough to remove the last traces of pre-existing base from the protein preparations used.

Equation (C) and (D) have generally been accepted and the value of 16 mEq/L inserted in the usual cation-anion diagrams for plasma or serum is based on them. Table I summarizes the results obtained in the experiments of Van Slyke and co-workers. The ACE values were calculated for pH 7.35 and expressed in mEq per 100 g protein. This was done because in our experiments to be discussed later all values have been expressed per 100 g protein. The pH 7.35 was selected because generally not arterial but venous blood is analysed which in the resting forearm appears to have a pH of on the average 7.35.

It should be stressed that Van Slyke et al. did their experiments at 38°C. The observations of Strauer et al. (1925) on the effect of temperature on acid-base-protein equilibrium in the pH range 7.00-7.70 suggest that whereas the molecular buffer value of the plasma proteins is not affected when the temperature changes from 38°C to 20°C, the apparent TEP increases by 0.02 on the average for each centigrade decrease in temperature. Accordingly the value of NCE should be about 3.5 mEq/100 g protein lower at 20°C than at 38°C, other conditions remaining equal.

In view of the accuracy needed the experiments of Van Slyke are not easily repeated and it is therefore not surprising to find that only two other attempts have been made to determine the NCE of the plasma proteins.

In 1929 DARROW & HARTMANN pointed out that equation (B) of Van Slyke and co-workers could not be used to calculate base binding power in patients in whom the A/G ratio was abnormal. They did therefore some experiments with beef serum which had been dialysed for 2 to 3 days against distilled water in an atmosphere of CO<sub>2</sub> and in the cold. During the dialysis part of the globulins precipitated.

After dialysis CO<sub>2</sub> titration after the manner of Van Slyke et al. was carried out separately on horse serum, on the washed precipitated globulins and on the proteins not precipitated by dialysis. Total protein as determined by the macro-Kjeldahl method using a factor of 6.25. Albumin and globulin were determined in the same way after fractionation by 22.2 per cent ammonium sulfate. Apparently two samples are studied, both at two pH values (about 7.2 and about 7.5).

(for the pH range covered by the experiments)

$$BP = 0.068 P_s (\text{pH} - 4.80) \quad (A)$$

where BP represents base bound by protein (*i.e.* NCE) in mEq and  $P_s$  serum protein in grams (The pH at which BP would be zero according to the equation - in this case 4.80 - will be indicated as apparent IEP). According to equation (A) the NCE of 100 g of normal horse protein is 17.4 mEq at pH 7.35 and increases by 6.8 mEq for every 1.0 unit increase in pH. It is interesting to note that the values used in the general equations from which the authors calculated the Donnan ratios across the erythrocyte membrane and across the capillary wall are based on this equation.

In 1927 in a paper concerned with the distribution of electrolytes between transudates and serum HASTINGS *et al* (1927) pointed out that equation (A) correlated BP with total protein only and that - as the titration curves of albumin and globulin differed greatly and moreover the albumin/globulin ratio was not the same in man as in the horse - the equation could not be used to calculate the 'base bound' by human serum proteins. They mentioned experiments in which the method described above was applied to dialysed human serum. From these experiments the following relation was calculated for human serum

$$BP = 0.097 P_s (\text{pH} - 5.26) \quad (B)$$

Equation (B) indicates a NCE of 20.3 mEq per 100 g of (normally constituted) human serum at a pH of 7.35 increasing with 9.7 mEq for every 1.0 pH unit increase. The experimental data from which equation (B) was derived have never been published as far as we could ascertain.

Instead another equation was proposed in 1928 by VAN SLYKE *et al*. It is based on the results obtained with albumin and globulin fractions of horse serum. The globulin was salted out from serum with half saturated  $(\text{NH}_4)_2\text{SO}_4$  albumin with saturated  $(\text{NH}_4)_2\text{SO}_4$  and both fractions were freed from small electrolytes by pressure dialysis against distilled water which had been saturated with  $\text{CO}_2$  to bring its reaction near to the isoelectric point of the protein fractions. In this study the protein solutions were not only titrated with  $\text{CO}_2$  but with HCl also. For that purpose the protein fractions were dissolved in enough NaOH to render the solution distinctly alkaline and then titrated electrometrically with 0.1 N HCl. The amount of cation opposed by protein valences was found by subtracting the HCl added from the NaOH originally present in the solution.

In three experiments 2 preparations of albumin (protein concentration about 50 g/Kg  $\text{H}_2\text{O}$ ) were studied, and in another three experiments 2 preparations of globulin (protein concentration 70.9 and 36.2 g/Kg  $\text{H}_2\text{O}$ ). The Na concentration was between 135 and 139 mEq/Kg  $\text{H}_2\text{O}$  and all experiments were done at 38°C. Determinations were done at 3 to 7 different pH values, ranging from 5.20 to 9.26, but in the final analysis the authors have only included the values obtained at pH values between 6.50 and 7.80. In the calculation of  $\text{HCO}_3^-$  a value of 6.11 was assumed for pK of carbonic acid.

The relation between pH and BP was again a linear one for both protein fractions and no difference was found between the results obtained with  $\text{CO}_2$  and with HCl titration. From their results the following equation was calculated for horse serum

$$BP = 0.124 \text{ Alb} (\text{pH} - 5.16) + 0.077 \text{ Glob} (\text{pH} - 4.89) \quad (C)$$

In their publication the protein values were expressed in grams of N. To make the equation comparable with the older ones we have expressed it in terms of grams of protein assuming an N factor of 6.25 as this was the factor which Van Slyke used for calculating equation (D).

In normal human serum Van Slyke *et al* found with their fractionation technique the ratio albumin-globulin to be 1:1. From (C) they calculated therefore for normal human serum the equation

$$BP = 0.104 P (pH - 5.08) \quad (D)$$

According to (D) the 'base binding power' that is the NCE, of 100 g human serum protein (at pH 7.35 and 38°C) is 23.6 mEq and increases 10.4 mEq per unit pH.

The correctness of 'extrapolating' the values found with horse serum proteins to human serum was tested with one sample of pooled human serum which had been dialysed for three months at 0°C. It contained 17.2 g/Kg H<sub>2</sub>O albumin and 14.4 g/Kg H<sub>2</sub>O globulin. The CO<sub>2</sub> titration was carried out at 4 different values of pH (6.01 to 7.97), the Na concentration was 136 mEq/Kg H<sub>2</sub>O and the temperature 38°C. At every pH the BP found was 5-7% lower than the value calculated from equation (D). At the end of their article Van Slyke *et al* commented on the fact that the earlier equations indicated a considerably lower value for BP. They concluded that probably in the earlier experiments dialysis had not been pushed far enough to remove the last traces of pre-existing base from the protein preparations used.

Equation (C) and (D) have generally been accepted and the value of 16 mEq/L inserted in the usual cation-anion diagrams for plasma or serum is based on them. Table 1 summarises the results obtained in the experiments of Van Slyke and co-workers. The NCE values were calculated for pH 7.35 and expressed in mEq per 100 g protein. This was done because in our experiments to be discussed later all values have been expressed per 100 g protein. The pH 7.35 was selected because generally not arterial but venous blood is analysed which in the resting forearm appears to have a pH of on the average 7.35.

It should be stressed that Van Slyke *et al* did their experiments at 38°C. The observations of S. Aoki *et al* (1925) on the effect of temperature on 'acid-base-protein equilibrium' in the pH range 7.00-7.70 suggest that whereas the molecular buffer value of the plasma proteins is not affected when the temperature changes from 38°C to 20°C, the apparent IEP increases by 0.02 on the average for each centigrade decrease in temperature. Accordingly the value of NCE should be about 3.5 mEq/100 g protein lower at 20°C than at 38°C, other conditions remaining equal.

In view of the accuracy needed the experiments of Van Slyke are not easily repeated and it is therefore not surprising to find that only two other attempts have been made to determine the NCE of the plasma proteins.

In 1929 DARROW & HARTMANN pointed out that equation (B) of Van Slyke and co-workers could not be used to calculate 'base binding power' in patients in whom the A/G ratio was abnormal. They did therefore some experiments with beef serum which had been dialysed for 2 to 3 days against distilled water in an atmosphere of CO<sub>2</sub> and in the cold. During the dialysis part of the globulins precipitated.

After dialysis CO<sub>2</sub> titration after the manner of Van Slyke *et al* was carried out separately on whole serum, on the washed precipitated globulins and on the proteins not precipitated by dialysis. Total protein is determined by the micro-Kjeldahl method using a factor of 6.25. Albumin and globulins are determined to the same way after fractionation by 22.2 per cent dioxonium sulfate. Apparently two samples are studied, both at two pH values (about 7.2 and about 7.5).

(for the pH range covered by the experiments)

$$BP = 0.068 P_s (\text{pH} - 4.80) \quad (A)$$

where BP represents base bound by protein (*i.e.* NCE) in mEq and  $P_s$  serum protein in grams (The pH at which BP would be zero according to the equation - in this case 4.80 - will be indicated as apparent IEP\*) According to equation (A) the NCE of 100 g of normal horse protein is 17.4 mEq at pH 7.35 and increases by 6.8 mEq for every 1.0 unit increase in pH. It is interesting to note that the values used in the general equations from which the authors calculated the Donnan ratios across the erythrocyte membrane and across the capillary wall are based on this equation.

In 1927 in a paper concerned with the distribution of electrolytes between transudates and serum HASTINGS *et al* (1927) pointed out that equation (A) correlated BP with total protein only and that - as the titration curves of albumin and globulin differed greatly and moreover the albumin/globulin ratio was not the same in man as in the horse - the equation could not be used to calculate the base bound by human serum proteins. They mentioned experiments in which the method described above was applied to dialysed human serum. From these experiments the following relation was calculated for human serum

$$BP = 0.097 P (\text{pH} - 5.26) \quad (B)$$

Equation (B) indicates a NCE of 20.3 mEq per 100 g of (normally constituted) human serum at a pH of 7.35 increasing with 9.7 mEq for every 1.0 pH unit increase. The experimental data from which equation (B) was derived have never been published as far as we could ascertain.

Instead, another equation was proposed in 1928 by VAN SLYKE *et al*. It is based on the results obtained with albumin and globulin fractions of horse serum. The globulin was salted out from serum with half saturated  $(\text{NH}_4)_2\text{SO}_4$ , albumin with saturated  $(\text{NH}_4)_2\text{SO}_4$  and both fractions were freed from small electrolytes by pressure dialysis against distilled water which had been saturated with  $\text{CO}_2$  to bring its reaction near to the isoelectric point of the protein fractions. In this study the protein solutions were not only titrated with  $\text{CO}_2$  but with HCl also. For that purpose the protein fractions were dissolved in enough NaOH to render the solution distinctly alkaline and then titrated electrometrically with 0.1 N HCl. The amount of cation opposed by protein valencies was found by subtracting the HCl added from the NaOH originally present in the solution.

In three experiments 2 preparations of albumin (protein concentration about 50 g/kg  $\text{H}_2\text{O}$ ) were studied, and in another three experiments 2 preparations of globulin (protein concentration 70.9 and 36.2 g/kg  $\text{H}_2\text{O}$ ). The Na concentration was between 535 and 139 mEq/kg  $\text{H}_2\text{O}$  and all experiments were done at 38°C. Determinations were done at 3 to 7 different pH values, ranging from 5.20 to 9.26, but in the final analysis the authors have only included the values obtained at pH values between 6.50 and 7.80. In the calculation of  $\text{HCO}_3^-$  a value of 6.11 was assumed for  $\text{pK}_1$  of carbonic acid.

The relation between pH and BP was again a linear one for both protein fractions and no difference was found between the results obtained with  $\text{CO}_2$  and with HCl titration. From their results the following equation was calculated for horse serum

$$BP = 0.124 \text{ Alb} (\text{pH} - 5.16) + 0.077 \text{ Glob} (\text{pH} - 4.89) \quad (C)$$

In their publication the protein values were expressed in grams of N. To make the equation comparable with the older ones we have expressed it in terms of grams of protein assuming an N factor of 6.25 as this was the factor which Van Slyke used for calculating equation (D).

In normal human serum Van Slyke *et al* found with their fractionation technique the ratio albumin-globulin to be 1.6. From (C) they calculated therefore for normal human serum the equation

$$BP = 0.104 P (\text{pH} - 5.08) \quad (D)$$

According to (D) the "base binding power" that is the NCE, of 100 g human serum protein (at pH 7.35 and 38°C) is 23.6 mEq and increases 10.4 mEq per unit pH.

The correctness of extrapolating the values found with horse serum proteins to human serum was tested with one sample of pooled human serum which had been dialysed for three months at 0°C. It contained 17.2 g/Kg H<sub>2</sub>O albumin and 14.4 g/Kg H<sub>2</sub>O globulin. The CO<sub>2</sub> titration was carried out at 4 different values of pH (6.01 to 7.97), the Na concentration was 136 mEq/Kg H<sub>2</sub>O and the temperature 38°C. At every pH the BP found was 5-7 / lower than the value calculated from equation (D). At the end of their article Van Slyke *et al* commented on the fact that the earlier equations indicated a considerably lower value for BP. They concluded that probably in the earlier experiments dialysis had not been pushed far enough to remove the last traces of pre-existing base from the protein preparations used.

Equation (C) and (D) have generally been accepted, and the value of 16 mEq/L inserted in the usual cation-an on diagrams for plasma or serum is based on them. Table 1 summarises the results obtained in the experiments of Van Slyke and co-workers. The NCE values were calculated for pH 7.35 and expressed in mEq per 100 g protein. This was done because in our experiments to be discussed later all values have been expressed per 100 g protein. The pH 7.35 was selected because generally not arterial but venous blood is analysed, which in the resting forearm appears to have a pH of on the average 7.35.

It should be stressed that Van Slyke *et al* did their experiments at 38°C. The observations of S. ADAM *et al* (1925) on the effect of temperature on acid-base-protein equilibrium in the pH range 7.00-7.70 suggest that whereas the molecular buffer value of the plasma proteins is not affected when the temperature changes from 38°C to 20°C, the apparent IEP increases by 0.02 on the average for each centigrade decrease in temperature. Accordingly the value of NCE should be about 3.5 mEq/100 g proteins lower at 20°C than at 38°C, other conditions remaining equal.

In view of the accuracy needed the experiments of Van Slyke are not easily repeated and it is therefore not surprising to find that only two other attempts have been made to determine the NCE of the plasma proteins.

In 1929 DARROW & HARTMANN pointed out that equation (B) of Van Slyke and co-workers could not be used to calculate "base binding power" in patients in whom the A/G ratio was abnormal. They did therefore some experiments with beef serum which had been dialysed for 2 to 3 days against distilled water in an atmosphere of CO<sub>2</sub> and in the cold. During the dialysis part of the globulins precipitated.

After dialysis CO<sub>2</sub> titration after the manner of Van Slyke *et al* was carried out separately on whole serum, on the washed precipitated globulins and on the proteins not precipitated by dialysis. Total protein was determined by the macro-Kjeldahl method using a factor of 6.25. Albumin and globulin were determined in the same way after fractionation by 22.2 per cent ammonium sulfate. Apparently ten samples were studied, both at two pH values (about 7.2 and about 7.5).



The globulins precipitated during dialysis were assumed to be representative for all globulins and from the  $\text{CO}_2$  titration of the precipitated fraction the following equation was obtained for bovine globulin

$$B \text{ Glob} = 0.04 \text{ Glob (pH} - 5.58)$$

where B Glob stands for base binding power of the globulins (in mEq) and Glob for grams of globulin. With the aid of the above equation and knowing the concentration of albumin and globulin as well as the base binding power of (dialysed) whole serum the following equation was obtained for bovine albumin

$$B \text{ Alb} = 0.141 \text{ Alb (pH} - 5.42)$$

The equations given by Darrow and Hartmann were rearranged by us in the notation used by Van Slyke and co workers. The NCE values calculated by means of these equations are presented in table 1

No details concerning the experiments are given but it is mentioned that after dialysis the serum still contained some calcium. The authors also pointed out that the equations are probably not correct because of the assumption of equal value of base binding power for precipitated and non precipitated globulin, and because of the subtractions necessary to obtain the equations for albumin. No data are given for the normal A/G ratio found with the fractionation technique used. But if we assume the ratio to be the same as that found by VAN SLYKE *et al* in 1928 (*i.e.* 1.6) we calculate from the equations given by Darrow and Hartmann a value of 19.5 mEq/100 g protein for the NCE of normal bovine serum protein. This is very nearly the value obtained for human serum from equation (B) of Van Slyke and coworkers.

HENDERSON *et al* (1931) have applied the same method (*i.e.*  $\text{CO}_2$  titration) to 10 samples of human plasma obtained from 6 normal persons. But their results are sparsely documented and lend themselves only to the calculation of the buffer value as the authors have assumed an arbitrary value for NCE at pH 7.40. They calculate for the pH range 7.0 to 7.8 a mean buffer value of 10.9 (expressed in mEq increase per 100 g protein per unit pH) (table 1)

*It is at times difficult to understand what precisely the authors have been doing, as practically no experimental data are given and only a composite graph and table are reproduced. Furthermore no mention is made of how the plasma was obtained and in what manner it was further treated. It is not even clear whether the samples were subjected to dialysis prior to the titration experiments.*

The results of the titration curves obtained for purified albumin (chapter I) can also supply information on the buffer value only. Recent data on bovine serum albumin (TANFORD *et al* 1955) suggest that at an ionic strength of 0.15 the buffer value in the pH range 7.0 to 8.0 is about 12.0 when expressed in mEq per 100 g albumin per unit pH. This value we derived from the titration curve published by Tanford and it is therefore admittedly an estimate, but it agrees rather well with the buffer value found by Van Slyke and co workers for horse albumin which was 12.4 (equation (C)). In a similar manner we calculated from KEPES' titration curves for bovine serum albumin a buffer value of approximately 11.0 in the pH range 7.0 to 7.5 (titration in the presence of 29 mEq/L KCl). For the globulins no such titration data exist.

The above mentioned data suggest that in so far as the buffer value is concerned

equations (C) and (D) of Van Slyke and co-workers are probably near the truth. For the absolute value of NCE no determinations are available more recent than those published by Van Slyke *et al* in 1928

Objections to the application of the results obtained by Van Slyke *et al* to human plasma. While reading the publications of Van Slyke and associates, especially the one from 1928, one is impressed by the detailed presentation of the experimental data and the obviously high accuracy of the analytical techniques. But there are several objections to generalising the results of these experiments

(a) The number of protein preparations studied was very small indeed and nothing is therefore known about the physiological variation.

(b) The final equation was derived from experiments on horse protein, and in the one instance in which it was tested with human serum preparations the latter had been dialysed for 3 months at 0°C.

(c) According to present knowledge the technique used for fractionating the proteins is a very rough one, both in the sense that the fractions obtained are not comparable to electrophoretically homogeneous albumin and globulin, and in the sense that the treatment might easily have damaged the protein molecules.

(d) The titrations were done in a solution of physiological ionic strength, but containing univalent cations only. Since the interaction of proteins with bivalent ions is quite different, it remains to be proven whether the results obtained with a solution containing Na as only cation are applicable to plasma without restriction. From the observations of Kepes discussed in chapter I one must conclude that this is not so.

(e) On the assumption that Na was the only cation present in the solution, Van Slyke *et al* could equate the known amounts of Cl and  $\text{HCO}_3^-$  against a known total amount of cations and therefore calculate the amount of cations opposing the only other anion present, *i.e.* protein. However as discussed in chapter I, protein also figures on the cationic side of the ion balance. In reality therefore the cation-anion balance was  $[\text{Na}^+] + [\text{Pr}^+] = [\text{Pr}^-] + [\text{Cl}^-] + [\text{HCO}_3^-]$  and since this equation contains two unknown quantities — the cation and the anion equivalency — it can not be solved. This stresses again the fact that the values found by Van Slyke *et al* refer to the *net* cation equivalency.

(f) Finally although only NCE was determined, the values given by Van Slyke are tacitly accepted as indicating net negative charge. The difference has been pointed out in chapter I.

#### ESTIMATION OF NCE FROM THE VALUES REPORTED FOR THE NORMAL PLASMA CONCENTRATIONS OF CATIONS AND ANIONS OTHER THAN PROTEIN

Theoretically for any plasma sample NCE of plasma protein can be derived from the difference between the sum concentration of all cations other than protein and that of all anions other than protein. In practice no accurate results are obtained for several reasons. Firstly the precise contribution of organic acids to the ionic balance is not known. But as it is small compared with the NCE of the proteins, this need not be a serious objection. A reasonable estimate of its magnitude should be possible from the normal values published for lactate, citrate, ketonbodies, non-esterified fatty acids and

amino acids. The fact that on the cation-side the ammonium ions are neglected is without influence since even in pathological conditions their total amount is never more than 0.1 mEq/L. The second difficulty encountered is more serious and is due to the errors in chemical determination. A positive error of 1% in the Na determination and a negative error of 1% in Cl analysis cumulate in overestimating NCE by something like 15%. The high accuracy achieved by Van Slyke *et al* in their experiments is partly due to the fact that the concentrations of Na and Cl were accurately known, as the solutions on which the determinations were done were prepared by the authors.

It should nevertheless be possible to estimate with the aid of the normal values published for cations and anions in plasma whether Van Slyke *et al*'s most recent value for NCE is of the right order of magnitude. With this end in mind table 2 was compiled.

#### Presentation of data in table 2

In principle we have only listed data which are documented reasonably well, but a few undocumented ones have also been included either because of the authority of the authors (GAMBLE, PETERS) or because they belong to the small number of investigations in which the cations as well as the anions were determined on the same plasma samples (JEANNERET *et al* 1954). Because they are always quoted the values given by Gamble in his classic lecture syllabus on the composition of the extracellular fluid head the list although we have been unable to find the experimental data on which they rest.

For historical reasons it must be mentioned that SCHWARTZ (1890), apparently the first to determine Na and Cl in human serum, found in the two normal sera analysed a Na concentration of 148.0 respectively 138.0 mEq/L and a Cl concentration of 100.0 respectively 102.4 mEq/L. For K values of about 8.0 mEq/L were found, presumably as a result of damage to the erythrocytes during preparation of the serum.

The weight of the values listed in table 2 is roughly indicated by the number of asterisks in the last column of the table. No asterisk signifies that the values were reported without, or with scant documentation; one asterisk indicates a reasonable degree of documentation; two asterisks detailed documentation. In this respect special attention has been given to Na and Cl. For Na (and for total base) a variety of analytical methods have been used. These are indicated in table 2. Cl was nearly always determined by one of the modifications of the open Carius method, the exception being the study by FAWCETT & WYNN who made use of potentiometric titration.  $\text{HCO}_3^-$  has always been calculated from total  $\text{CO}_2$  and pH (where the latter was generally assumed to be 7.34).

Concerning the composition of table 2 the following must be said. On the side of the cations we have listed separately the concentration of the individual cations, the sum of these concentrations and the concentration of total base. Total base has been determined more frequently than the sum concentration of the individual cations. Moreover in calculating the latter some authors had to assume a value for the concentration of Mg. In that case the value listed for the sum concentration of the cations has been placed between brackets.

On the side of the anions the concentrations of Cl, of  $\text{HCO}_3^-$  and of inorganic phosphate are listed with the sum concentration of Cl +  $\text{HCO}_3^-$ .  $\text{SO}_4^{2-}$  and organic acids were not determined in the studies mentioned but an estimate of the normal concentration of these substances can be made from the literature (table 3).

*Rest anions.* It would appear that the average normal value for plasma  $\text{SO}_4$  concentration is 0.7 mEq/L, and that normally the organic acids contribute about 3.0 mEq/L to the anionic valencies in plasma and nothing to the cationic valencies. The value of 3.0 is based on the assumption that the sum of the equivalent concentrations of all anionic "trace" substances is about 1.0 mEq/L. The amino acids were listed with these "trace" anions because at normal plasma pH their positive and negative valencies will nearly balance each other (the normal plasma concentration of filterable amino acids is only about 2.5 mM/L (WALTON *et al.* 1962)).

As the normal plasma concentration of inorganic phosphate was on the average 2.2 mEq/L in the studies listed in table 2, we have estimated that inorganic phosphate, sulfates and organic acid together (the so-called *rest anions* or *RA*) contribute on the average 6 mEq/L to the anionic valencies in normal plasma.

We have therefore subtracted from the values listed under the heading (cat minus Cl +  $\text{HCO}_3$ ) in table 2 the value of 6.0 in order to obtain an estimate of NCE. The resulting values have been listed in the corresponding column.

In the cation-anion diagram of normal plasma used by Gamble in his famous lectures on extracellular fluid, the value of NCE is given as 18 mEq/L, i.e. the value calculated with the aid of equation (D) of Van Slyke and co-workers. The concentration of organic acids is given by Gamble as 6.0 mEq/L since this amount made the total anionic valency in Gamble's diagram equal to the total cationic valency. However if we consider NCE as the unknown quantity and assume the contribution of the organic acids to be 3.0 mEq/L (see before), we calculate from the concentrations which Gamble reports for the other ions, a NCE value of 19.0 mEq/L. This is the value listed in table 2.

#### Discussion of the data presented in table 2

The first fact which becomes apparent on studying the contents of table 2 is the small number of studies in which a sufficiently large number of the various cations and anions were determined to allow an estimate of NCE to be made. Furthermore the three studies in which Na and K were determined by flame photometry are either sparsely documented (DANOWSKI *et al.* 1955; ANGIO & REILMAN 1959) or not at all (JEANNERET *et al.* 1954).

The second fact is the large variation in the values reported for the normal plasma or serum Na concentration even after the introduction of the flame photometer. The variation in the reported values for total base is smaller but the values still cover a range of about 10 mEq/L. The values reported for Cl on the other hand have been fairly constant throughout the years. Clearly a reasonable estimate of NCE can only be made from those studies in which cations (or total base) and anions were both determined in the same plasma or serum samples. In ten of the reported studies this appears to have been the case. Seven of these were published before 1940. But by assuming for Mg a normal plasma value of 2.0 mEq/L, the results of JEANNERET *et al.* (1954), of DANOWSKI *et al.* (1955) and of ANGIO & REILMAN (1959) could also be used for the calculation of NCE. (From the series of DANOWSKI *et al.* only the values for adults were taken. The Cl and  $\text{HCO}_3$  values for males and for females have been averaged).

If we consider the NCE values obtained from these selected series, we find that they vary from 10 to 25 mEq/L plasma whereas the protein concentration only varies from 65.0 to 74.8 g/L. The large spread in NCE is apparently a result of the large spread in the values found for the sum concentrations of the cations. Furthermore it can be seen that in the studies published since 1930 the value estimated for NCE is in general below the value expected from equation (D) of Van Slyke and co-workers. Several authors have commented on the discrepancy.

amino acids. The fact that on the cation-side the ammonium ions are neglected is without influence, since even in pathological conditions their total amount is never more than 0.1 mEq/L. The second difficulty encountered is more serious and is due to the errors in chemical determination. A positive error of 1% in the Na determination and a negative error of 1% in Cl analysis cumulate in overestimating NCE by something like 15%. The high accuracy achieved by Van Slyke *et al* in their experiments is partly due to the fact that the concentrations of Na and Cl were accurately known, as the solutions on which the determinations were done, were prepared by the authors.

It should nevertheless be possible to estimate with the aid of the normal values published for cations and anions in plasma whether Van Slyke *et al*'s most recent value for NCE is of the right order of magnitude. With this end in mind table 2 was compiled.

### Presentation of data in table 2

In principle we have only listed data which are documented reasonably well, but a few undocumented ones have also been included either because of the authority of the authors (GAMBLE, PETERS) or because they belong to the small number of investigations in which the cations as well as the anions were determined on the same plasma samples (JEANNERET *et al* 1954). Because they are always quoted the values given by Gamble in his classic lecture syllabus on the composition of the extracellular fluid head the list, although we have been unable to find the experimental data on which they rest.

For historical reasons it must be mentioned that SCHMIDT (1850) apparently the first to determine Na and Cl in human serum, found in the two normal sera analysed a Na concentration of 148.0 respectively 138.0 mEq/L and a Cl concentration of 100.0 respectively 102.4 mEq/L. For K values of about 8.0 mEq/L were found, presumably as a result of damage to the erythrocytes during preparation of the serum.

The weight of the values listed in table 2 is roughly indicated by the number of asterisks in the last column of the table. No asterisk signifies that the values were reported without or with scant, documentation; one asterisk indicates a reasonable degree of documentation; two asterisks detailed documentation. In this respect special attention has been given to Na and Cl. For Na (and for total base) a variety of analytical methods have been used. These are indicated in table 2. Cl was nearly always determined by one of the modifications of the open Carius method; the exception being the study by FAWCETT & WYNN who made use of potentiometric titration.  $\text{HCO}_3^-$  has always been calculated from total  $\text{CO}_2$  and pH (where the latter was generally assumed to be 7.35).

Concerning the composition of table 2 the following must be said. On the side of the cations we have listed separately the concentration of the individual cations, the sum of these concentrations and the concentration of total base. Total base has been determined more frequently than the sum concentration of the individual cations. Moreover in calculating the latter some authors had to assume a value for the concentration of Mg. In that case the value listed for the sum concentration of the cations has been placed between brackets.

On the side of the anions the concentrations of Cl, of  $\text{HCO}_3^-$  and of inorganic phosphate are listed with the sum concentration of Cl +  $\text{HCO}_3^-$ ,  $\text{SO}_4^{2-}$  and organic acids were not determined in the studies mentioned but an estimate of the normal concentration of these substances can be made from the literature (table 3).

They determined Na (by modification of the arsanyl zinc acetate method), K, Ca,  $\text{HCO}_3$ , Cl,  $\text{PO}_4$ , total protein and protein fractions and calculated the NCE with the aid of equation (C) of Van Slyke *et al.* Mg was assumed to be 2 mEq/L. For every serum sample they calculated the difference between the sum concentration of the cations on the one hand and the sum concentration of Cl,  $\text{HCO}_3$ , phosphate and (calculated) NCE on the other and the values thus obtained were plotted against the globulin content of the serum.

The results are shown in fig. 8. It can be seen that the cation-anion difference - which ought to represent  $\text{SO}_4$  + organic acids - ranged from 4.0 to -0.5 mEq/L for the 10 normal persons investigated, leaving the somewhat low average value of about 2 mEq for the sum concentration of  $\text{SO}_4$  and organic acids. More impressive however is the

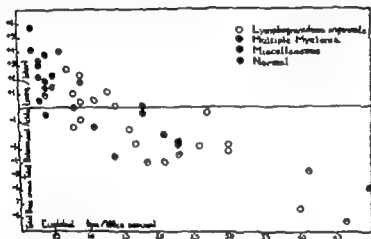


Fig. 8. Correlation between (total base minus total determined acids) and globulin content of serum. See text for explanation. Reproduction of fig. 1 in publication of OJERU *et al.* (1934) *J. clin. Invest.* 15: 473

evident negative correlation which existed between the amount of globulin per 100 cc serum and "total base minus total determined acids" the latter assuming rather large negative values at high concentrations of globulin. The authors concluded that obviously equation (C) of Van Slyke *et al.* cannot be used when the protein composition is quantitatively and qualitatively pathological. In the diseases studied the globulin fraction evidently had a far lower NCE than the globulin fraction studied by Van Slyke *et al.*

Possible causes for the observed variation in the estimated values of NCE

(a) The large variation in the values reported for normal plasma Na concentration and total base

From table 2 it will be seen that the Na values obtained with the pyroantimonate method are rather high, with the notable exception of those reported by Marrack. The values found with the gravimetric arsanyl zinc acetate method are somewhat lower and vary from 135.0 to 142.5 some authors reporting consistently low values. The controversy between Hald and Consolazio *et al.* has already been mentioned. Contrary to what

ATCHLEY & BENEDICT (1930) determined total base, chloride, bicarbonate and phosphate in the serum of 10 normal adults and of a number of patients. They found the value for total base rather low in comparison to the values published earlier. Inserting for NCE the value calculated with the aid of equation (D) into the cation-anion diagram for serum they observed that in the normal persons studied on the average only 0.1 mEq/L plasma was left for  $\text{SO}_4$  and all organic anions together. No conclusion was drawn as to the validity of equation (D) but in the introduction to their experimental results they stated that the equations (*i.e.* for calculating base bound by the proteins) are based on data so difficult to obtain that they undoubtedly will be altered by further study.

PETERS called attention to the fact that in certain patients with the nephrotic syndrome the concentration of Cl in the serum was abnormally high and the estimated sum of anionic equivalents (which included NCE calculated with the aid of equation (D)) exceeded the observed concentration of total base. He postulated that part of the serum Cl was loosely bound by the serum lipids. He has tried to determine this lipid-chlorine by extracting the serum lipids with hot alcohol and ether (PETERS & MAN 1934). In normal persons the extracts contained 2.7 to 5.7 mEq/L Cl and in patients with increased serum lipids this amount could be as high as 11 mEq. But the correlation between lipid-chlorine concentration and lipid content of the plasma was a very rough one, as was the correlation between the concentration of lipid-chlorine and apparent base deficit. Peters pointed out that the methodical difficulties were such that no quantitative conclusions were possible and he promised further experiments on this interesting phenomenon. However no further evidence has emerged and to our knowledge lipid chlorine has not been mentioned in later publications.

The conclusion of Peters that in a number of persons the cation-anion diagram of plasma indicates apparently a cation deficit or anion excess (*i.e.* when for NCE the value calculated with the aid of equation (C) or equation (D) was inserted) was based on the analyses done by HALD. She developed a modification of the Stadel and Ross method for the total base determination, and a rather intricate fractionation procedure to obtain Na from ashed serum, the Na being determined gravimetrically after precipitation with uranyl zinc acetate. Her normal values (1933) for total base and Na were considerably lower than those published before that time, with the exception of those found by SUMNERMAN (1930). The validity of her results has been questioned by CONSOLAZIO & TALBOTT (1940). They found distinctly higher values for total base (with electrodeialysis and with the iodometric total base determination of VAN SLYKE *et al.* (1927)), and for Na (with a modification of the uranyl zinc acetate method). But on the same samples, the method of HALD gave in their hands practically the same low values as Hald had found herself. They concluded that with Hald's technique Na was co-precipitated during the preliminary precipitation of phosphate and they virtually proved their contention in 1941 (CONSOLAZIO & DRILL 1941). Hald could not confirm this and concluded from experiments with  $^{22}\text{Na}$  that no Na got lost during phosphate precipitation (1946). However from her experimental data it is evident that some radioactivity was found in the precipitated phosphate and in the same publication she reported normal Na values, which are about 2 mEq/L higher than those she published in 1933 and in 1937 (HALD & EISENMANN 1937). Even so they remain low in comparison to most other values published including the ones later obtained with flame photometry.

That in a number of patients the cation-anion diagram apparently indicates an anion excess, if equation (C) or (D) of Van Slyke *et al.* are used for calculating NCE, was clearly demonstrated by GUTMAN *et al.* (1936). They studied the acid base equivalency of the serum in patients with hyperproteinæmia due to a very high euglobulin content of the serum (Howe's method was used for the protein fractionation).

The 'protein interference effect' has also been observed for K (BERNSTEIN 1952, BERGSTRÖM & HULTMAN 1962, BERGMAN *et al.* 1963).

BERNSTEIN has apparently been the first to observe differences between the flame photometer values obtained with plasma and with deproteinised plasma. He attributed this to an increase in the volume occupied by protein as a result of trichloroacetic acid binding by the proteins.

The above cited observations apparently point to the value obtained for deproteinised plasma as representing the true Na concentration. But it must be doubted whether this conclusion is correct.

SACKNER & SUNDERMAN (1958) report very low Na values although they analysed deproteinised plasma (Beckman flame photometer). On the other hand among the highest normal Na values reported are those found by BERNSTEIN (1952) and DIANEWICKI *et al.* (1955) who analysed plasma. The validity of the results obtained with neutron activation analysis is questionable (V. LUTZMAN *et al.* 1963). The observation reported by BERGMAN *et al.* (1963) the spread found for  $^{24}\text{Na}$  is nearly three as large as that found for  $^{23}\text{Na}$ . Moreover if the values obtained for  $^{24}\text{Na}$  are correct the average plasma Na concentration of the 14 normal subjects studied by Bergman *et al.* would have been rather high, i.e. 143.4 mEq/L. Furthermore Bergström and Hultman appear not to have corrected the values obtained for deproteinised plasma for the effect of protein removal on the concentration of the remaining serum constituents.

Clearly further experiments are necessary to decide whether analysis of deproteinised plasma (or serum) gives more accurate results than the analysis of plasma (or serum) directly.

From this survey it appears that there is no common operator which can explain the differences between the values reported for normal plasma Na concentration. It is therefore unjustifiable to average the various values, and we must conclude that the accurate value of the Na concentration in normal plasma or serum is still a matter of conjecture.

The values reported for total base lead to a somewhat similar conclusion. But there the variation is less and the only two authors who have reported remarkably low values are HALD and SUNDERMAN. With SACKNER the latter has published in 1958 the results of a study in which the Na concentration has been determined both with the gravimetric uranyl zinc acetate method and with the flame photometer and the total base concentration by 4 methods including Sunderman's procedure for measuring conductivity (1945) and Hald's method. Only 5 normal sera were investigated but in these and in the sera of 21 patients with a variety of diseases, low values for the concentration of both Na and total base were found with all methods used. However the agreement between the conductivity procedure and Hald's method is not wholly unexpected, since the first appears to have been standardised against the latter (SUNDERMAN 1942). Since Sunderman's results are not so well documented as those of Hald or Consolazio an explanation of the difference with the values reported by others is not feasible.

#### (b) Variations in condition of subject at moment of sample taking

The results obtained by the various authors may have been influenced by the condition of the persons whose blood was studied. WALAAS & WALAAS (1949) found total base to increase some 5 mEq/L as a result of breaking fast by a (normal?) meal. On the other hand ARINO & RILMAN (1959) found only a slight increase in serum Na concentration after breakfast, whereas FAWCETT & WYNN (1956) saw no change after a meal.



might be expected the Na values obtained with the flame photometer show a larger instead of a smaller spread than the values obtained by chemical methods. This is partly due to the fact that after the introduction of the flame photometer the determination of Na and K seemingly became a simple matter. This has sometimes led to a somewhat uncritical acceptance of results which are definitely inferior to those obtained by previous workers with the more laborious techniques. ELLIOT & HOLLEY (1951) determined serum Na in a 1:50 dilution with a Perkin Elmer flame photometer using the method of MOSHER *et al* (1949). The latter added gelatin to their standard solutions, but not to the plasma and from their data it is evident that gelatin in the concentration used has a depressive effect on the flame photometer values obtained both for Na and for K. The standard solutions give therefore too low a galvanometer reading and as a consequence the plasma values are overestimated. The exceedingly high value reported by OVERMAN & DAVIS (1947) was obtained on protein free filtrate of plasma in a 1:60 dilution and also with a Perkin Elmer. The anticoagulant used is not mentioned and since some heparin preparations contain a very high concentration of Na this might have caused a positive error. The rather high value reported by DANOWSKI *et al* (1955) was obtained on serum using a Patwin flame photometer and a serum dilution of 1:250 (personal communication of DANOWSKI 1962). The intermediate values reported by JEANNERET *et al* (1954) are not documented. The value reported by TARAEL *et al* (1952) was obtained with a Barclay flame photometer using an internal standard and dilutions of 1:200 and 1:400. MOHUN & COOK (1962<sup>1</sup>) found the very low value of 135.0 for normal plasma samples in 1:500 dilution with the EEL flame photometer. The heparin used by them contained no Na. The very high dilution seems to preclude any disturbing influence due to viscosity and yet their values are very much lower than those of ROEGHOLT, who used a dilution of 1:25. In ROEGHOLT's study (1955) Na-containing heparin was used as anti-coagulant, but this contributed only 0.5 mEq/L to the reported Na value.

Of special interest are the observations of BERGSTRÖM & HULTMAN (1962) who determined Na concentration with an Eppendorf flame photometer both in plasma and in deproteinised plasma (protein precipitated with trichloroacetic acid). Plasma Na concentration was also determined by means of neutron activation analysis (BERGSTRÖM 1962). Flame photometer analysis of deproteinised plasma resulted in Na concentrations which were on the average 6.5 mEq/L higher than those found when analysing plasma. As the values found with neutron activation were in good agreement with those obtained for deproteinised plasma the authors concluded that the latter are correct. They suggested that the very low values reported by Mohun and Cook were due to a protein interference effect. The latter presumably varies according to the flame photometer used. In the ensuing discussion MOHUN & COOK (1962<sup>2</sup>) reported that they had subsequently found a protein interference effect of the same order as Bergström and Hultman. In their original experiments they had been misled by the fact that commercial control sera agreed precisely with their own standards, but had now observed that contrary to fresh serum, these control sera showed no protein interference effect. BERGMAN *et al* (1963) reported experiments in which  $^{24}\text{Na}$  was added to heparinised plasma and the latter then subjected to ultrafiltration. Plasma and ultrafiltrate were analysed both for  $^{24}\text{Na}$  (with a G-M tube) and for  $^{23}\text{Na}$  (with an Eppendorf flame photometer) and it was found that the ratio  $^{24}\text{Na}/^{23}\text{Na}$  was higher in plasma than in the ultrafiltrate. The authors concluded that this observation was further evidence for the protein interference effect.

The 'protein interference effect' has also been observed for K (BERNSTEIN 1952, BERGSTROM & HULTMAN 1962, BERGMAN *et al* 1963).

BERNSTEIN has apparently been the first to observe a difference between the flame photometer values obtained with plasma and with deproteinised plasma. He attributed this to an increase in the volume occupied by proteins as a result of trichloroacetic acid binding by the proteins.

The above cited observations apparently point to the value obtained for deproteinised plasma as representing the true Na concentration. But it must be doubted whether this conclusion is correct.

SACKNER & SUNDERMAN (1958) report very low Na values although they analysed deproteinised plasma (Beckman flame photometer). On the other hand among the highest normal Na values reported are those found by BERNSTEIN (1952) and DAWOWSKI *et al.* (1955) who analysed plasma. The validity of the results obtained with neutron activation analysis is questionable (VAN LERUWEN *et al* 1963). In the observation reported by BERGMAN *et al.* (1963) the spread found for  $^{24}\text{Na}$  is nearly twice as large as that found for  $^{22}\text{Na}$ . Moreover if the values obtained for  $^{24}\text{Na}$  are correct the average plasma Na concentration of the 14 normal subjects studied by Bergman *et al.* would be rather high, 143.4 mEq/L. Furthermore Bergstrom and Hultman appear not to have corrected the values obtained for deproteinised plasma for the effect of protein removal on the concentration of the remaining serum constituents.

Clearly further experiments are necessary to decide whether analysis of deproteinised plasma (or serum) gives more accurate results than the analysis of plasma (or serum) directly.

From this survey it appears that there is no common operator which can explain the differences between the values reported for normal plasma Na concentration. It is therefore unjustifiable to average the various values, and we must conclude that the accurate value of the Na concentration in normal plasma or serum is still a matter of conjecture.

The values reported for total base lead to a somewhat similar conclusion. But there the variation is less and the only two authors who have reported remarkably low values are HALD and SUNDERMAN. With SACKNER the latter has published in 1958 the results of a study in which the Na concentration has been determined both with the gravimetric cyanil zinc acetate method and with the flame photometer and the total base concentration by 4 methods including Sunderman's procedure for measuring conductivity (1945) and Hald's method. Only 5 normal sera were investigated but in these and in the sera of 21 patients with a variety of diseases, low values for the concentration of both Na and total base were found with all methods used. However the agreement between the conductivity procedure and Hald's method is not wholly unexpected, since the first appears to have been standardised against the latter (SUNDERMAN 194 ). Since Sunderman's results are not so well documented as those of Hald or Consolazio an explanation of the difference with the values reported by others is not feasible.

(b) *Variations in condition of subject at moment of sample taking*

The results obtained by the various authors may have been influenced by the condition of the persons whose blood was studied. WALAAS & WALAAS (1949) found total base to increase some 5 mEq/L as a result of breaking fast by a (normal?) meal. On the other hand AYONNO & RILMAN (1959) found only a slight increase in serum Na concentration after breakfast, whereas FAWCETT & WYNN (1956) saw no change after a meal

might be expected the Na values obtained with the flame photometer show a larger instead of a smaller spread than the values obtained by chemical methods. This is partly due to the fact that after the introduction of the flame photometer the determination of Na and K seemingly became a simple matter. This has sometimes led to a somewhat uncritical acceptance of results which are definitely inferior to those obtained by previous workers with the more laborious techniques. ELLIOT & HOLLEY (1951) determined serum Na in a 1:50 dilution with a Perkin Elmer flame photometer using the method of MOSHER *et al* (1949). The latter added gelatin to their standard solutions but not to the plasma and from their data it is evident that gelatin in the concentration used has a depressive effect on the flame photometer values obtained both for Na and for K. The standard solutions gave therefore too low a galvanometer reading and as a consequence the plasma values are overestimated. The exceedingly high value reported by OVERMAN & DAVIS (1947) was obtained on protein free filtrate of plasma in a 1:60 dilution and also with a Perkin Elmer. The anticoagulant used is not mentioned and since some heparin preparations contain a very high concentration of Na this might have caused a positive error. The rather high value reported by DANOWSKI *et al* (1955) was obtained on serum using a Patwin flame photometer and a serum dilution of 1:250 (personal communication of DANOWSKI 1962). The intermediate values reported by JEANNERET *et al* (1954) are not documented. The value reported by TARAIL *et al* (1952) was obtained with a Barclay flame photometer using an internal standard and dilutions of 1:200 and 1:400. MOHUN & COOK (1962) found the very low value of 135.0 for normal plasma samples in 1:500 dilution with the EEL flame photometer. The heparin used by them contained no Na. The very high dilution seems to preclude any disturbing influence due to viscosity and yet their values are very much lower than those of Roegholt, who used a dilution of 1:25. In ROEGHOLT's study (1955) Na-containing heparin was used as anti-coagulant, but this contributed only 0.5 mEq/L to the reported Na value.

Of special interest are the observations of BERGSTRÖM & HULTMAN (1962) who determined Na concentration with an Eppendorf flame photometer both in plasma and in deproteinised plasma (protein precipitated with trichloroacetic acid). Plasma Na concentration was also determined by means of neutron activation analysis (BERGSTRÖM 1962). Flame photometer analysis of deproteinised plasma resulted in Na concentrations which were on the average 6.5 mEq/L higher than those found when analysing plasma. As the values found with neutron activation were in good agreement with those obtained for deproteinised plasma the authors concluded that the latter are correct. They suggested that the very low values reported by Mohun and Cook were due to a protein interference effect. The latter presumably varies according to the flame photometer used. In the ensuing discussion MOHUN & COOK (1962) reported that they had subsequently found a protein interference effect of the same order as Bergström and Hultman. In their original experiments they had been misled by the fact that commercial control sera agreed precisely with their own standards, but had now observed that, contrary to fresh serum, these control sera showed no protein interference effect. BERGMAN *et al* (1963) reported experiments in which  $^{24}\text{Na}$  was added to heparinised plasma and the latter then subjected to ultrafiltration. Plasma and ultrafiltrate were analysed both for  $^{24}\text{Na}$  (with a G-M tube) and for  $^{23}\text{Na}$  (with an Eppendorf flame photometer) and it was found that the ratio  $^{24}\text{Na}/^{23}\text{Na}$  was higher in plasma than in the ultrafiltrate. The authors concluded that this observation was further evidence for the protein interference effect.

lend themselves to a more accurate interpretation. The one study in which a definite attempt was made to check the validity of equation (C) of Van Slyke *et al* in cases with pathological hyperproteinaemia (GUTMAN *et al* 1936) has already been mentioned. It clearly indicated that pathological globulins may have a very low NCE.

In 1945 BROCH published a study on the regulation of serum electrolytes in which he presented observations on total base (determined by electrodialysis), Cl,  $\text{HCO}_3$  and serum protein concentration in a total of 99 patients suffering from a variety of diseases (among which all sorts of febrile and infectious diseases, liver disease, and cardiac insufficiency). In about half the number of patients serum was analysed more than once in the course of the illness. The most remarkable finding was that in a number of patients large and often sudden changes in total base were seen resulting in much lower values than those found in health. Since the values for Cl and  $\text{HCO}_3$  remained fairly constant, Broch assumed that large and sudden decreases in NCE were responsible. Apparently its value could become zero or even negative (when a value of 9 mEq/L was accepted for the concentration of the rest anions). Broch explained this peculiar phenomenon by assuming sudden changes in IEP of the plasma proteins in the direction of normal blood pH. In one patient with liver cirrhosis the difference between total base on the one hand and the sum concentration of Cl,  $\text{HCO}_3$ , and inorganic phosphate on the other was found to be 11.7 mEq. But since the albumin concentration was 25.8 g/L, and the globulin concentration 96.6 g/L (method of Howe), Broch calculated from formula (C) of Van Slyke an expected value for NCE of 25.4. This patient obviously belongs to the group of patients studied by Gutman *et al*. But in practically all the other patients observed by Broch no such protein abnormalities were present. The fact that in these patients NCE seemed to alter considerably within a few days has led the author to the conclusion that this is the expression of a regulatory mechanism which counteracts changes in plasma Na concentrations by changing the degree of dissociation of the proteins. No suggestion is made as to the manner in which these abrupt changes in dissociation are brought about (BROCH 1953).

The presentation and discussion of the technique of electrodialysis with which the values for total base concentration are obtained is rather sketchy and it is to be deplored that apparently no attempt has been made to verify at least some of the very low values found for total base by means of summation of the concentrations of the individual anions.

But apart from the sudden changes observed, the values given by Broch indicate that on the whole the NCE of the plasma proteins was lower in the patients than in his normal persons.

In 1947 HALD *et al* published a study in which they considered the possibility to estimate the concentration of serum Na from the sum concentration of Cl and  $\text{HCO}_3$ . The material used for this purpose was gathered over a period of 15 years and included both Na values obtained with the method of Hald and with the flame photometer. In normal persons they found the difference between Na concentration and the sum concentration of Cl and  $\text{HCO}_3$  to be on the average +5.0 mEq/L, but in all patients studied (42, among whom patients with adrenal insufficiency, diabetic acidosis and severe uraemia) the difference was larger. This contrasts with the findings of Broch, but different types of disease were studied in the two groups.

Summarizing it can be concluded that apart from the well documented observations of

But the authors last mentioned did observe that about half an hour after the meal the concentration of Cl usually fell about 2 mEq/L and total CO<sub>2</sub> content increased by a similar amount.

Apparently sex has no influence on the value found for normal plasma Na concentration or total base (DANOWSKI 1955, ROEHOLT 1955, FAWCETT & WYNN 1956) and the difference originally observed by SALVESEN (1928) has therefore not been corroborated. However the plasma concentration of Cl appears to be somewhat higher in the female than in the male and bicarbonate concentration somewhat lower (HALD *et al* 1947, DANOWSKI *et al* 1955, FAWCETT & WYNN 1956).

As to age the investigations by DANOWSKI *et al* (1955) suggest that the normal value for plasma Na concentration changes somewhat in the course of life. But in most of the studies represented in table 2 the subjects were in the age group 20-50 year and differences in age cannot therefore explain the observed spread in the values reported for normal plasma or serum Na.

### CONCLUSION

The publication of VAN SLYKE *et al* in 1928 remains the latest source of information on the absolute value of NCE of the plasma proteins. An attempt to verify their validity for human plasma with the aid of published data on the difference between the concentration of small cations and of small anions in serum or plasma meets with the difficulty that the normal values reported for Na (and for total base) vary considerably. There are some indications that possibly the NCE of the plasma proteins is smaller than the value calculated with the aid of the last reported equation of Van Slyke *et al* but for the moment it cannot be decided whether this is due to an error in the determination of the cation-anion balance, or to the fact that the data of Van Slyke are not applicable to human plasma.

### NCE OF PLASMA PROTEINS (PATHOLOGICAL PLASMA)

Because of the tendency of some plasma proteins to associate with ions and with a number of molecular substances, a large increase in the plasma concentration of the latter (as seen in uraemia or hyperlipaemia) may well result in a change in NCE of the plasma proteins. The effect of adding longchain fatty acids to serum on the electrophoretic characteristics of albumin and lipoproteins (GORDON 1955, also GOODMAN 1958) is rather suggestive in this respect. But because of the analytical difficulties involved very little is known about the NCE in disease beyond the fact that a change in the albumin-globulin ratio does affect its magnitude.

A number of authors have studied the cation-anion balance in plasma in such condition as pneumonia, diabetic acidosis, uraemia, nephrosis, etc. (MARRACK 1923, ATCHLEY *et al* 1923, 1930, SUNDERMAN *et al* 1926, SUNDERMAN 1930, BLACKFAN & HAMILTON 1927, PETERS *et al*, 1929<sup>1</sup>, 1929<sup>2</sup>, SALVESEN 1928, GUTMAN *et al* 1936). It is difficult to draw from these data conclusions concerning the NCE of the plasma proteins because the concomitant changes in the concentrations of phosphate, sulphate and organic acids introduce an error which in some cases becomes of the same magnitude as NCE itself. The data obtained for diseases in which only the proteins show pathological changes

After LOEB (1922) introduced the concept of the Donnan equilibrium in biology a large number of investigators have determined the distribution of biologically important ions across all sorts of living and artificial membranes.

Originally it was thought that this phenomenon could explain the movement of the electrolytes through the membranes but it soon became apparent that the Donnan distribution could only function as a transport mechanism if another mechanism created a condition of constant disequilibrium. Furthermore the Donnan distribution could not explain the transport of equally charged ions in opposite directions. As result clinical interest in the Donnan equilibrium flagged. Recently interest has returned, one of the reasons being that it provides the means to determine to what extent filtrable ions are complex-bound by the macro-molecular substances present in cartilage and connective tissue (see for instance FARMER & SCHURER 1957).

#### DISTRIBUTION OF THE MAIN FILTRABLE IONS BETWEEN PLASMA (OR SERUM) AND NATURAL (OR SYNTHETIC) INTERSTITIAL FLUID

##### Selection from data reported in the literature

For our purpose only results obtained with plasma or serum are of interest. A further selection had to be made with regard to the membrane which has been studied. The results obtained from the study of special compartments of the interstitial space (cerebrospinal fluid, intraocular fluid, synovial fluid) will not be considered because of the possibility or even certainty (cerebrospinal fluid), that the separating membrane is not really inert with regard to the passage of the various ions. The reader is referred to MANERY's admirable review on water and electrolyte metabolism (1954), in which the author presents and critically discusses the available data concerning the concentration ratios between plasma and other body fluids. Here we will limit ourselves to experiments in which ion distribution has been studied *in vitro* by subjecting normal plasma or serum to equilibrium dialysis or to filtration under pressure, and to *in vivo* studies concerning the distribution of the ions between serum (generally obtained from venous forearm blood) and transudates (*i.e.* edema, pleural fluid or peritoneal fluid). The results of both groups of studies are summarised in table 4. Since we are discussing in this section the findings with normal plasma or serum the results obtained in the clinical studies are in fact somewhat out of place here. They refer to patients in whom the plasma albumin concentration must have been rather low. The authors have generally not determined the protein composition and it is therefore not possible to use these data for a study of the effect of changes in protein composition on the magnitude of the concentration ratios. Since the difference between the protein concentrations on both sides of the semipermeable membrane is the decisive factor in the distribution of the ions, we have followed Manery in excluding from our review those observations in which the transudate contained more than 10 g/L of protein. But even so the difference between the protein concentration on both sides of the membrane is in the clinical studies considerably less than in the *in vitro* experiments. This, together with the fact that in the clinical studies hypoalbuminaemia must have been present in most cases, leads one to expect that the concentration ratios observed in the clinical studies must on the average have been higher than those to be expected for the equilibrium between normal plasma and normal interstitial fluid. Notwithstanding these objections we have included the clinical observations in our review because they still provide information concerning the relative magnitudes of the concentration ratios of the different ions. But the results

Gutman *et al* no data are available from which a definite conclusion can be reached as to the NCE of the plasma proteins in various diseases.

## DISTRIBUTION OF THE MAIN FILTRABLE IONS BETWEEN NORMAL PLASMA OR SERUM AND (NATURAL OR SYNTHETIC) INTERSTITIAL FLUID AND CONCLUSIONS CONCERNING THE INTERACTION OF THESE IONS WITH NORMAL PLASMA PROTEINS

In the case of plasma (and serum) the study of the effect of protein on the ability of the filtrable ions to pass a semipermeable membrane has provided much information on ion protein interaction. RONA & TAKAHASHI (1911) dialysed serum from a number of animals against various solutions containing 0.9% NaCl but different concentrations of  $\text{CaCl}_2$ . It was assumed that the salt solution in which the Ca concentration had not changed after this compensation dialysis contained Ca in a concentration equal to the concentration of freely filtrable Ca in the serum. From these experiments Rona and Takahashi concluded that about 25-35% of serum Ca is prevented from passing through a semipermeable membrane because it has formed a complex with serum proteins. A large number of studies has since been published in which the same criterium was used to determine whether a filtrable ion was bound by the plasma proteins. All authors agreed that a considerable proportion of serum Ca was not free to filtrate and CUSHNY (1920) and TSCHIMBER *et al* (1924) found the same for Mg. Within the (large) error of analysis the Na and Cl in serum appeared to be freely filtrable (VAN CREVELD 1921, RICHTER-QUITNER 1922, NEUHAUSEN & PINCUS 1923, TSCHIMBER *et al* 1924) and RONA & PETOW (1923) concluded from dialysis experiments in neutral and acid solutions that about 14% of serum Na was associated with proteins in a salt-type manner. For K the evidence was conflicting. RICHTER-QUITNER (1922) found about 60% of serum K to be non filtrable. RONA & PETOW (1923) found a smaller but not negligible quantity of non-dialysable K but NEUHAUSEN & PINCUS (1923) concluded that all serum K was filtrable. However in the early experiments factors such as the lack of refined analytical methods, neglect of pH influence and neglect of correction for volume occupied by proteins all operated to preclude an accurate interpretation of the observed behaviour of the univalent cations. Moreover the Donnan equilibrium as a cause for unequal distribution of filtrable ions was not fully appreciated until some years later.

Yet presumably the best method at present available for studying ion protein interaction in a complicated solution such as plasma is to determine the concentrations of the various filtrable ions both in plasma (or serum) and its filtrate (or dialysate) and to compare the concentration ratios observed with the Donnan ratio expected. The principle has been extensively used by NORTHROP & KUNITZ in the study of ion-gelatin interaction and has already been explained in chapter I. If the Donnan ratio is known the amount of ion bound in a complex type manner can be calculated. If the Donnan ratio is not known at least a qualitative estimate of ion-protein interaction can be made by comparing the concentration ratios of the various ions. A study of the literature concerning the distribution of ions across semipermeable membranes separating plasma or serum from a protein free filtrate or dialysate can therefore provide information concerning the nature of ion-protein interaction in plasma.

In the second part of table 4 the results of the *in vivo* studies are summarised. It has already been pointed out that these have only a relative value for the study of conditions in normal plasma. A further restriction must be made with regard to the values obtained for  $R_{Cl}$  and  $R_{HCO_3}$ . In the case of these two ions the concentration differs significantly according to whether serum from arterial or from (cubital) venous blood is analysed. It is still a matter of conjecture which of the two is most like the blood that is in equilibrium with the transudate. Both DARROW *et al* and GILLIGAN *et al* took arterial and venous blood samples. They found that the analysis of arterial blood resulted for  $R_{Cl}$  in a value about 0.5 / higher but for  $R_{HCO_3}$  in a value about 5-10 / lower than that obtained when venous blood was analysed. Gilligan *et al* supposed that the mean of the two values is near the true value for the equilibrium between blood and transudate.

We must conclude that in the *in vivo* studies in which venous blood was sampled the values obtained for  $R_{HCO_3}$  are definitely too high when compared with the values of  $R$  obtained for the other ions. For  $R_{Cl}$  the error is negative but so small as to be negligible in comparison to all other errors made.

#### Discussion of data presented in table 4

The low values found for the concentration ratio in the case of the bivalent cations and the high value found in the case of inorganic phosphate differ significantly from the ratios found for the univalent ions. As to the latter there appears to be a distinct difference between the values found for  $R_{Cl}$  and  $R_{HCO_3}$  and those found for the concentration ratios of univalent cations. In the case of the latter the ratio is consistently somewhat lower than in the case of the univalent anions.

From table 4 it is evident that for normal plasma (at normal pH)  $R_{Cl}$  is the most constant of the ratios and on the average between 0.97 and 0.98 both in the *in vitro* and in the *in vivo* studies. For  $R_{HCO_3}$  the range of reported values is considerably larger. The average value is between 0.99 and 1.00, but only two *in vitro* studies are available and since in the *in vivo* studies generally venous blood was analysed, it must be doubted whether the difference with  $R_{Cl}$  is real. The small number of values reported for the concentration ratio of inorganic phosphate are all higher than 1.0.

As to the cations in the case of Na and K the interpretation of the data is made difficult by the fact that in the early *in vitro* experiments and all clinical studies a gravimetric method of analysis was used. But at the same time the earlier studies were mostly concerned with animal sera, the later studies with human serum. From table 4 it can be seen that in the earlier studies a lower value was found for  $R_{Na}$  than in the later experiments in which a flame photometer was used. Whatever the explanation may be, for Na the average value of the concentration ratio was about 0.94 in the *in vitro* studies on human serum, although the most recent publication (SALAMEN 1961) reports a somewhat lower value. The values obtained with animal sera are on the whole considerably lower with the exception of the values found by SWAN *et al* (1956), who studied dogs, and those found by DAVSON *et al* (1949) and DAVSON (1955), who studied rabbits, cats and dogs. Whereas Swan used flame photometry DAVSON used a gravimetric Na determination as well as measurement of  $^{23}Na$ . In the *in vitro* studies only chemical methods of analysis were used and  $R_{Na}$  was on the average 0.95. At least part of the difference with those *in vitro* studies in which also a gravimetric method of Na determina-



obtained in the *in vitro* experiments are undoubtedly nearer the values to be expected for normal plasma or serum.

#### Manner of presenting the data in table 4

In order to make the concentration ratios comparable with the Donnan ratio – as it is usually expressed – the concentration ratios are in the case of the cations obtained by dividing the outside concentration by the inside concentration in the case of the anions by dividing inside by outside concentrations. For the bivalent ions the concentration ratio *per se* is given and not the square root of the concentration ratio

The square root derives from the theory of membrane equilibria, which is concerned with activities. A considerable quantity of bivalent cations is protein-bound in a complex-type manner and their concentration ratios are therefore certainly not equal to their activity ratios. As a consequence the use of the square root is not correct. Apparently this has not always been realised (see for instance MAHELY 1954)

In table 4 the *in vitro* studies are mentioned first. The data were obtained either by equilibrium dialysis of serum against artificial interstitial fluid or by filtering serum under pressure and comparing concentrations in the ultrafiltrate with those in the serum. With the second method no real equilibrium is established since the protein concentration increases continuously during the production of the filtrate. INGRAHAM *et al* (1932) have therefore used the average of the concentration in the original serum and the concentration in the residue for the calculation of the inside concentrations. TARAIL *et al* (1952) used the original serum concentrations. It is therefore to be expected that the concentration ratios reported by Tarail *et al* will be higher than those reported by Ingraham *et al*

In the experiments of GREENE & POWER (1931) an ingenious method of *in vivo* dialysis was used. In both legs the femoral artery and vein were connected by a tube of semipermeable material (not specified) with a sac on the outside containing a modified Ringer solution. By using on one side a slightly hypotonic and on the other side a slightly hypertonic solution it could be seen when equilibrium was reached. The concentration then found in the artificial interstitial fluid was compared with the concentration in serum obtained from femoral vein blood.

In most of the studies presented in table 4 Na and K were analysed by chemical methods. In the studies in which Na and K were determined by flame photometry serum or plasma was analysed. The pH was about 7.40 in all studies except in that of Ingraham *et al* who experimented at pH  $\pm$  7.00 and  $\pm$  7.70. Greene and Power mention that in experiments with *in vitro* dialysis considerable changes in the concentration ratios of Cl and Na were observed when the pH changed from 6.2 to 8.2, but no details are given.

The studies included in table 4 were selected because they contain data concerning the distribution of univalent ions. But the number of studies in which at the same time the concentration ratios of the Ca and the Mg ions were determined is so small that we have included in table 4 the data of WALSER (1961) which are the most recent ones concerning the bivalent cations.

$R_c$  and  $R_a$  were calculated by us from the data given in Table I of Walser's publication on the assumption that water content of normal plasma is (990–0.8 P) g per litre, P indicating g protein per litre plasma (VAN SLIKE *et al* 1923; GREENE & POWER 1931). The pH value at 37°C was calculated with the aid of Rosenthal's correction factor for temperature effect (see chapter III)

In the second part of table 4 the results of the *in vivo* studies are summarised. It has already been pointed out that these have only a relative value for the study of conditions in normal plasma. A further restriction must be made with regard to the values obtained for  $R_{Cl}$  and  $R_{NaCl}$ . In the case of these two ions the concentration differs significantly according to whether serum from arterial or from (cubital) venous blood is analysed. It is still a matter of conjecture which of the two is most like the blood that is in equilibrium with the transudate. Both DARROW *et al* and GILLIGAN *et al* took arterial and venous blood samples. They found that the analysis of arterial blood resulted for  $R_{Cl}$  in a value about 0.5 / higher but for  $R_{NaCl}$  in a value about 5-10 / lower than that obtained when venous blood was analysed. Gilligan *et al* supposed that the mean of the two values is near the true value for the equilibrium between blood and transudate.

We must conclude that in the *in vivo* studies in which venous blood was sampled, the values obtained for  $R_{NaCl}$  are definitely too high when compared with the values of  $R$  obtained for the other ions. For  $R_{Cl}$  the error is negative, but so small as to be negligible in comparison to all other errors made.

#### Discussion of data presented in table 4

The low values found for the concentration ratio in the case of the bivalent cations and the high value found in the case of inorganic phosphate differ significantly from the ratios found for the univalent ions. As to the latter there appears to be a distinct difference between the values found for  $R_{Cl}$  and  $R_{NaCl}$  and those found for the concentration ratios of univalent cations. In the case of the latter the ratio is consistently somewhat lower than in the case of the univalent anions.

From table 4 it is evident that for normal plasma (at normal pH)  $R_{Cl}$  is the most constant of the ratios and on the average between 0.97 and 0.98 both in the *in vitro* and in the *in vivo* studies. For  $R_{NaCl}$  the range of reported values is considerably larger. The average value is between 0.99 and 1.00 but only two *in vitro* studies are available and since in the *in vivo* studies generally venous blood was analysed, it must be doubted whether the difference with  $R_{Cl}$  is real. The small number of values reported for the concentration ratio of inorganic phosphate are all higher than 1.0.

As to the cations in the case of Na and K the interpretation of the data is made difficult by the fact that in the early *in vitro* experiments and all clinical studies a gravimetric method of analysis was used. But at the same time the earlier studies were mostly concerned with animal sera, the later studies with human serum. From table 4 it can be seen that in the earlier studies a lower value was found for  $R_K$  than in the later experiments in which a flame photometer was used. Whatever the explanation may be for Na the average value of the concentration ratio was about 0.94 in the *in vitro* studies on human serum, although the most recent publication (SALMÖNEN 1961) reports a somewhat lower value. The values obtained with animal sera are on the whole considerably lower with the exception of the values found by SWAN *et al* (1956), who studied dogs, and those found by DAVSON *et al* (1949) and DAVSON (1955), who studied rabbits, cats and dogs. Whereas Swan used flame photometry DAVSON used a gravimetric Na determination as well as measurement of  $^{22}Na$ . In the *in vivo* studies only chemical methods of analysis were used and  $R_{Na}$  was on the average 0.95. At least part of the difference with those *in vitro* studies in which also a gravimetric method of Na determina-

tion was used can be explained by the lower protein concentrations prevalent in the *in vivo* studies

For  $R_K$  the earlier *in vitro* studies report remarkably low values, but in the studies in which the flame photometer was used  $R_K$  was found to be about the same as  $R_{Na}$ . Notable exceptions are the lower ratios reported by FOLK *et al* (1948) and by SALMINEN (1961). From their data no explanation can be found for this deviating value. Indeed both TARAIL *et al* (1952) and FOLK *et al* (1948) have commented on the fact that in the course of time the ratios reported for K have gradually risen and they conclude that the old values must have been the result of technical errors, a conclusion in which they are joined by MANERY.

As was to be expected, the lowest ratios are found for Ca. Those reported for Mg though somewhat higher are of the same order of magnitude. The fact that  $R_{Ca}$  was found considerably higher in the *in vivo* studies than in the *in vitro* experiments is presumably due to the differences in protein concentration and composition between the two series of observations.

Concerning the effect of pH on the magnitude of the concentration ratios, only the observations of Ingraham *et al* are available. They suggest that a - physiologically speaking - large change in pH apparently has a rather small effect on  $R_{Cl}$  and  $R_x$ .

#### THE DONNAN RATIO FOR THE EQUILIBRIUM BETWEEN PLASMA AND INTERSTITIAL FLUID

The data compiled in table 4 suggest that the differences in the values obtained for the concentration ratios of the various ions are real. This makes it more difficult to make an accurate estimate of the value of the Donnan ratio. The inaccuracy to be expected in using pH measurement for determining the Donnan ratio was already pointed out in chapter I and the  $H^+$ -ion concentration ratio found by HASTINGS *et al* (1927) cannot seriously be considered as representing the Donnan ratio for normal plasma. To our knowledge no further values have been published. As mentioned in chapter I SALMINEN (1961) has determined the membrane potential in his ultrafiltration studies. But the average value which he found for normal serum (*i.e.* 9.3 mV) corresponds with a Donnan ratio of about 0.69 - a value which is clearly impossible in view of the magnitude of the concentration ratios reported for Na and for Cl.

An indirect estimate of the Donnan ratio has been made by VAN SLYKE, WU & MCLEAN (1923) who derived an equation by which the Donnan ratio could be calculated from the sum concentration of the filtrable anions and from the base binding power of the proteins on both sides of the semipermeable membrane. If no protein is present in the filtrate or dialysate the equation simplifies to (for historical reasons we have used the notation of Van Slyke *et al*)

$$r = \frac{[A]_p}{[A]_f + [BP]_f}$$

where  $[A]_p$  is the sum concentration in serum of all anions other than protein and  $[BP]_p$  is NCE of proteins. Assuming with GAMBLE (1950) that for normal plasma at pH 7.40  $[A]_p = 150$  mEq/Kg  $H_2O$  and  $[BP]_p = 17$  mEq/kg  $H_2O$  we obtain for  $r$  a value of 0.949. In chapter I we have derived a similar equation and there we pointed out the

difficulties to be expected when the equation is used without due attention to ion activities. But since we are here engaged in a historical survey we will restrict ourselves to pointing out that according to Van Slyke *et al* the Donnan ratio at the capillary wall must be about 0.950 under normal conditions. This estimate is based on the assumption that normally the interstitial fluid contains negligible amounts of non filtrable ionic substances.

#### AMOUNT OF ION PROTEIN BOUND IN A COMPLEX TYPE MANNER IN NORMAL PLASMA

With the above estimate of the value of  $r$  in mind, the results represented in table 4 appear to indicate that in normal plasma small amounts of Cl and of  $\text{HCO}_3$  (about 2-3 %) are bound in a complex type manner by the plasma proteins. For inorganic phosphate this amount appears to be about 15 %. When we consider the values obtained with the aid of the flame photometer the activity of the Na-ions appears to be only slightly decreased by plasma proteins, that of K-ions somewhat more. The Ca-ions show a decrease of about 30-40 % in their activity which is in fair agreement with the results obtained with the frog heart method (McLEAN & HASTINGS 1935) and the blood coagulation method (SOULIER & CROCHER 1958). In the studies reported in table 4  $R_{Mg}$  has seldom been determined. The most one can say is that according to table 4 the activity of Mg-ions is somewhat less decreased by plasma protein than the activity of the Ca-ions.

For the univalent ions the above conclusions are compatible with the data obtained in experiments with purified albumin (see chapter I). GREENE & POWER (1931) calculated from their *in vitro* dialysis experiments that about 7 % of serum Na and 20 % of serum K was not dissociated, but as the Cl-ratio was assumed to indicate the Donnan ratio and the concentration ratios for Na and K are about the lowest reported, their conclusion appears to be in error. Actually in experiments with dogs, in which ascites fluid was compared with serum, GREENE *et al* (1931) found both for Na and K a ratio of 0.94 (table 4) at a serum protein concentration only 20 % lower than in the *in vitro* dialysis experiments. However the studies of Greene and Power concerned dog serum and since they have been done in a very careful manner their results have to be seriously considered. Table 4 demonstrates that there are no recent *in vitro* studies comparable in scope to the studies of HASTINGS *et al* and GREENE & POWER. Furthermore only in the studies in which the flame photometer was used a ratio of about 0.94 was found for Na, and in the study by SWAN *et al* the Na and K ratios were, so to speak, by-products of an investigation of the distribution of sulphate ions. In view of the fact that the normal Na concentration of plasma is still a matter of debate as a result of the very different values found by authors who all used flame photometer analysis, confirmation of the findings of TARJIL *et al* SWAN *et al* and SALMINEN is needed before the results of the earlier investigators can be disregarded. Especially the observation that the flame photometer gives different values for Na and K concentration according to whether plasma or deproteinised plasma is analysed has serious consequences for the interpretation of the concentration ratios and the calculation of protein-bound ion

tion was used can be explained by the lower protein concentrations prevalent in the *in vivo* studies

For  $R_K$  the earlier *in vitro* studies report remarkably low values, but in the studies in which the flame photometer was used  $R_K$  was found to be about the same as  $R_{Na}$ . Notable exceptions are the lower ratios reported by FOLK *et al* (1948) and by SALMINEN (1961). From their data no explanation can be found for this deviating value. Indeed, both TARAIL *et al* (1952) and FOLK *et al* (1948) have commented on the fact that in the course of time the ratios reported for  $K$  have gradually risen and they conclude that the old values must have been the result of technical errors, a conclusion in which they are joined by MANERY.

As was to be expected the lowest ratios are found for  $Ca$ . Those reported for  $Mg$ , though somewhat higher are of the same order of magnitude. The fact that  $R_{Ca}$  was found considerably higher in the *in vivo* studies than in the *in vitro* experiments is presumably due to the differences in protein concentration and composition between the two series of observations.

Concerning the effect of pH on the magnitude of the concentration ratios only the observations of Ingraham *et al* are available. They suggest that a - physiologically speaking - large change in pH apparently has a rather small effect on  $R_{Cl}$  and  $R_{Na}$ .

#### THE DONNAN RATIO FOR THE EQUILIBRIUM BETWEEN PLASMA AND INTERSTITIAL FLUID

The data compiled in table 4 suggest that the differences in the values obtained for the concentration ratios of the various ions are real. This makes it more difficult to make an accurate estimate of the value of the Donnan ratio. The inaccuracy to be expected in using pH measurement for determining the Donnan ratio was already pointed out in chapter I and the  $H^+$ -ion concentration ratio found by HASTINGS *et al* (1927) cannot seriously be considered as representing the Donnan ratio for normal plasma. To our knowledge no further values have been published. As mentioned in chapter I SALMINEN (1961) has determined the membrane potential in his ultrafiltration studies. But the average value which he found for normal serum (*i.e.* 9.3 mV) corresponds with a Donnan ratio of about 0.69, a value which is clearly impossible in view of the magnitude of the concentration ratios reported for  $Na$  and for  $Cl$ .

An indirect estimate of the Donnan ratio has been made by VAN SLYKE, WU & McLEAN (1923) who derived an equation by which the Donnan ratio could be calculated from the sum concentration of the filtrable anions and from the base binding power of the proteins on both sides of the semipermeable membrane. If no protein is present in the filtrate or dialysate the equation simplifies to (for historical reasons we have used the notation of Van Slyke *et al*)

$$r_{\text{an}} = \frac{[A]_s}{[A]_f + [BP]_f}$$

where  $[A]_s$  is the sum concentration in serum of all anions other than protein and  $[BP]_s$  is NCE of proteins. Assuming with GAMBLE (1950) that for normal plasma at pH 7.40  $[A] = 150 \text{ mEq/Kg H}_2\text{O}$  and  $[BP]_s = 17 \text{ mEq/Kg H}_2\text{O}$  we obtain for  $r$  a value of 0.949. In chapter I we have derived a similar equation and there we pointed out the

More recently PRASAD and coworkers have studied ultrafiltrable Ca in various disease states including acidosis and alkalosis. A review of their work was given by PRASAD in 1960. Similar but less comprehensive studies were published by FANCONI & ROSE (1958) TEREPKA *et al* (1958) and VAN LEEUWEN *et al* (1961). The general gist of these studies is that albumin has the highest affinity that normal  $\gamma$ -globulins have a lower affinity and that pathological  $\gamma$ -globulins show a distinctly lower tendency to form a complex association with Ca. As to the influence of pH fairly large changes are necessary before appreciable changes occur in the amount of Ca associated with protein (HOPKINS *et al* 1952, TORIBARA *et al* 1957, LOKEN *et al* 1960, PETERSON *et al* 1961, WALSER 1962). As in the present study our interest is mainly in NCE and in the univalent cations, we shall not attempt to discuss all these data, but one further study concerning Ca must be mentioned. GLUECK *et al* (1962) observed in two patients, one with multiple myeloma and the other with an unexplained dysproteinemia, a peculiar syndrome characterised by hyperglobulinaemia, clotting defects and osteoporosis. The most interesting finding was that, whereas total serum Ca was practically normal, ultrafiltrable Ca was definitely too low and they could show that in these patients a pathological globulin was present which bound Ca extensively.

For Mg the data of PRASAD *et al* (1961) indicate that albumin shows again the highest affinity. As to the other protein fractions no definite conclusions are possible from their work and no other data appear to exist. A rather peculiar phenomenon was reported by some authors, who found for Mg a very low ultrafiltrable fraction in patients with hyperthyroidism but a very high filtrable fraction in myxoedema (SOFFER *et al* 1939, 1941 and DOVE & LAVIETTE 1942). Their observations could not be confirmed by CORP & WOLFF (1942) and BASSELL (1945), and from the discussions by Soffer and by Dove it does not become clear how the observed phenomenon must be explained. The total Mg concentrations were not significantly different from normal and no data are given about protein composition and plasma lipids.

From this short survey it is evident that more observations and experiments are necessary to obtain accurate information concerning the interaction between protein and filtrable plasma ions in disease. Even in the case of Ca there is still need for a systematic study of plasma or serum containing pathological proteins. The observation of Glueck *et al* should be a warning that in certain diseases proteins with unexpected affinity for cations (or anions) may circulate.



### *Summary of chapter II*

The experiments with horse serum published in 1928 by Van Slyke *et al* represent still the most recent determination of the NCE of plasma proteins. According to it the value of NCE (at pH 7.35) is 23.5 mEq/100 g protein, or about 16.0 mEq/L for normal plasma.

There are objections against applying to human plasma the results obtained by Van

## CONCLUSION

In conclusion the following can be said. An accurate estimate of the Donnan ratio (for normal serum or plasma) is not available. But if we accept a value of 0.950 for the *in vitro* experiments listed in table 4 the differences between the concentration ratios found for the various filtrable plasma ions apparently indicate the existence of a complex type of interaction between Ca, Mg, Cl, phosphate and presumably also  $\text{HCO}_3$  on the one hand, and the plasma proteins on the other. In the case of Na and K the evidence is conflicting.

It would appear that further experiments are needed concerning the concentration ratio of the univalent cations and anions under conditions which are well defined as to protein concentration, pH, ionic strength and electrolyte composition. These experiments should also include a study of the relation between pH and concentration ratio as such data are extremely scarce.

## INTERACTION OF FILTRABLE IONS WITH PLASMA PROTEINS UNDER PATHOLOGICAL CONDITIONS

The number of studies concerning the univalent ions is very small indeed and limited to observations on the concentration ratio of Na and/or K in ultrafiltration experiments. Only in two of these studies both cations were analysed. TARAIL *et al* (1952) found a normal value for the concentration ratios when they averaged the results obtained with serum from 10 patients (among whom 4 with renal insufficiency and 4 with diabetes mellitus). But the range of values was considerably larger than in normal persons and the protein content and the protein composition are not reported. SALMINEN (1961) studied the serum of a much larger number of patients (suffering from hepatic cirrhosis, nephrosis, pulmonary tuberculosis or renal insufficiency) and determined among other things both the protein concentration and the protein composition. His main conclusion is that the albumin concentration of the serum is the most important factor determining the concentration ratios of Na and K. Ultrafiltration experiments with solutions containing purified protein fractions showed that at pH 7.40  $\gamma$  globulin does practically not affect the concentration ratios. One may conclude from his experiments that whereas at pH 7.40 albumin binds fairly large amounts of both Na and K – presumably in a salt type manner –  $\gamma$ -globulin has no affinity for these cations at physiological pH values.

More is known concerning the interactions between the serum proteins and the bivalent cations, in particular Ca. One of the earliest studies in patients was done by BENDIEN & SNAPPER (1933) who from their data concluded that Ca interacted with albumin only. Later studies proved that although most of the non filtrable fraction of serum Ca is indeed associated with albumin, some is also bound by the globulin fraction and GUTMAN & GUTMAN (1937) already suspected that differences in affinity for Ca existed within the group of globulins. CHU & HASTINGS (1938) concluded from *in vitro* dialysis experiments that the normal globulins have a greater affinity for Ca than the pathological  $\alpha$  globulin found in the diseases studied by Gutman and Gutman, *i.e.* multiple myeloma and lymphogranuloma inguinale. DRINKER *et al* (1939) using the frog heart method found that the globulin fractions which precipitate at the lowest salt concentration show a much smaller affinity for Ca than the fractions which precipitate at higher salt concentrations.

## PART II



Slyke *et al* which make it necessary to determine NCE in human plasma. The values reported for the ionic composition of normal human plasma were found no help in this respect, both because of the limited number of studies from which a reasonable estimate of NCE can be made (assuming the concentration of the rest anions 6.0 mEq/L in normal plasma) and because the estimates thus obtained vary from 10 to 25 mEq/L. This variation is apparently largely the result of the spread in the values reported for normal plasma Na concentration a spread which is not easily explained.

Concerning the value of NCE in disease, accurate quantitative data do not exist, and reliable estimates from the ionic composition of plasma are very scarce indeed.

As to the concentration ratios of the filtrable ions for the equilibrium between normal plasma (or serum) and natural (or synthetic) interstitial fluid, the number of studies is surprisingly small and the values obtained show a considerable spread. But it would appear that there is a real difference between the average ratios found for the univalent cations and those found for the univalent anions, suggesting that either or both are partially bound in a complex type manner. A definite conclusion is not possible since at present no accurate values are known for the Donnan ratio. Also data concerning the correlation between the pH and the concentration ratios of the plasma ions are virtually non-existent.

Concerning the protein binding of univalent ions in disease practically nothing is known in contrast to the numerous data which have been gathered with regard to the protein binding of Ca.

## *Chemical analysis*

The method adopted for the determination of the net cation equivalency of plasma proteins (chapter IV and chapter V) is rather susceptible to errors in analysis, since the results of at least seven different methods cumulate in the final value. For protein Na, Cl and  $\text{HCO}_3$  in particular a relatively small percentage error in analysis will result in a large error in NCE. Special attention will therefore be given to systematic and accidental errors in general and to those in the case of protein Na, Cl and  $\text{HCO}_3$  in particular.

### SYRINGES, GLASSWARE AND STOPPERS

Dry Luer Lok syringes were used throughout. After soaking in warm tap water they were brushed, vigorously rinsed with tap water, boiled for 20 minutes in distilled water and left to dry in sterile containers.

All glassware was kept separated from that used for routine determinations. After soaking in water and brushing, the glass tubes were stored in potassium dichromate sulfuric acid solution for at least 24 hours, then thoroughly rinsed with tap water and with distilled water and finally dried in a sterilizer.

For closure of the glass tubes rubber caps and rubber stoppers were used. After soaking and brushing they were boiled in distilled water, rinsed and dried.

An isotonic NaCl solution stored for 24 hours in stoppered glass tubes, cleaned in the manner described, failed to show any change in Na and Cl concentration.

### OBTAINING AND HANDLING OF SAMPLES

#### *In vivo ultrafiltration*

The samples were obtained from a cubital vein via an indwelling 18 gauge needle, which between sampling was fitted with a stylet. 14-18 ml blood was drawn in 10 ml syringes and immediately injected through a rubber cap into a round-bottom glass tube flushed with end-expiratory air just prior to the beginning of the experiment. The capacity of the tubes varied from 16 to 20 ml. Until 29-5-61 the tubes contained 10  $\mu$ l of a 5% heparin solution. Then quite suddenly late coagulation started to occur in some of the plasma samples and from 8-6-61 on the amount of heparin was increased to 30  $\mu$ l.

According to the manufacturer no change in the fabrication procedure had been made and the altered effectivity of the heparin solution may have been due to the aging of the batch.



Immediately after sampling the blood samples were placed for 4 minutes in a water bath with the indicated temperature so that they had the correct temperature before they were put into the thermostated centrifuges. The samples to be compared were centrifuged simultaneously. During centrifugation the temperature of the blood remained constant to within 1°C.

**Loss of CO<sub>2</sub>** - It is highly improbable that the 0.15 ml air contained in the dead space of the 10 ml syringes induced CO<sub>2</sub> loss during sampling, since drawing 2 ml air into a syringe containing 6 ml blood and subsequent gentle shaking did not alter the pH of the blood significantly. Nor did lowering the pressure in the syringe to about 400 mm Hg (table 8). This remarkable resistance to CO<sub>2</sub> loss may be partly explained by the fact that the blood cools on entering the syringe, which increases the solubility of the gas.

Flushing the rubber-capped test tubes with end-expiratory air was presumably adequate in preventing loss of CO<sub>2</sub> since no change in pH and only a slight change in Hb saturation were observed during the transfer of blood from the syringe and the subsequent tilting necessary to mix heparin and blood (table 9).

We conclude that the plasma on which the determinations were done represents 'true plasma' but for the addition of the heparin solution. A correction can be made for the latter (see chapter VI).

#### Equilibrium dialysis

10 ml Luer Lok syringes were used. First 1 ml of the sample was drawn with which the air of the dead space was expelled, and then the rest of the sample - 10 ml - was drawn. About 6-7 ml was injected into rubber stoppered test tubes, 3 ml remaining in the metal-capped syringe for determination of pH and total CO<sub>2</sub>. The pH of serum and of a bicarbonate buffered saline solution did not alter during 12 hours storage in the syringes. All chemical determinations were done within 7 hours after sample taking.

### CHEMICAL METHODS

All chemical determinations were done in duplicate, unless stated differently.

#### Water content

##### Technique

Glass stoppered weighing bottles were used (diameter 25 mm, height 25 mm). After cleaning they were stored in potassium dichromate sulfuric acid solution and before use thoroughly rinsed with distilled water.

After placing the weighing bottles in an electric steriliser at 108°C, constant weight was reached within 24 hours. Then a pipet, accurately calibrated to contain a volume of the order of 0.5 ml, was filled with the fluid to be investigated. The pipet was weighed before and after filling, the difference representing the wet weight of the sample. The latter was then delivered into the weighing bottle, several washings of distilled water being used to effect a complete transfer (by following this procedure both water content and the specific gravity of the sample could be calculated). The bottles were then placed again into the steriliser and 24 hours later they were reweighed after cooling to room temperature in an exsiccator. The difference between this weight and the weight of the bottle found before introduction of the sample represents the dry weight of the sample.

In the first experiments 3 ml blood of each sample was injected separately into a small rubber capped glass tube (containing 5  $\mu$ l 5 / heparin solution and end-expiratory air) for the determination of total CO<sub>2</sub>. We repeatedly tested CO<sub>2</sub> content and Cl concentration of both the small and the larger tube. They were always found to be identical within the errors of determination. A representative example is given in table 5. In later experiments the total sample was injected into the larger tube and after centrifugation 2 ml plasma was drawn anaerobically for CO<sub>2</sub> determination into 2 ml Luer Lok syringes which were closed with a metal cap after expelling the air contained in the dead space of the syringe. No loss of CO<sub>2</sub> could be detected during this procedure (table 6).

Within ten minutes after the blood samples were drawn the blood was centrifuged (8 min 3000 rpm at 22-24°C) and the plasma pipetted into dry rubber stoppered test tubes (in the later experiments after a sample was separated for total CO<sub>2</sub> determination in the manner described above).

Blood for determination of pH and percentage HbO<sub>2</sub> was drawn immediately after the large sample in a 5 ml Luer Lok syringe, the dead space of which ( $\pm 0.1$  ml) was filled with 5 / heparin solution. The metal capped syringes were placed in ice water.

All chemical determinations were done within 7 hours after sampling, but pH and HbO<sub>2</sub> were measured within 2 hours.

#### *Changes in the composition of plasma caused by the sampling and centrifugation of blood and the subsequent handling of plasma*

**THE ADDITION OF HEPARIN SOLUTION** - We used Thromboliquine Organon which contains 5 / heparin and the following amounts of ion (in mEq/L): Na 320-340, K 3-13, Cl 120-135, no Ca or Mg (3 phials from 2 different batches). The errors resulting from the addition of the heparin solution are small and will be discussed when the results of the *in vivo* ultrafiltration experiments are presented (chapter VI).

**HAEMOLYSIS.** This occurred only exceptionally and in the course of our experiments not more than 10 samples had to be rejected for this reason. When the aspect of a plasma sample was dubious its K concentration was used for a guide: when the latter was in keeping with the K concentrations found in the samples immediately preceding and following the suspected sample, the latter was accepted.

**EFFECT OF TEMPERATURE DURING CENTRIFUGATION** - ROSENTHAL (1948) observed a lower total CO<sub>2</sub> content and Cl concentration when plasma was obtained by centrifuging at room temperature instead of at 38°C. No experimental data are given. ASTRUP (1956) reports one experiment in which he observed a 2 / decrease in plasma CO<sub>2</sub> content when erythrocytes and plasma were separated at room temperature instead of at 38°C. C. MOLLER (1959) mentions that he observed a similar effect but gives no details. On the other hand SEVERINGHAUS *et al* (1956<sup>1</sup>) in well documented experiments found no difference in plasma CO<sub>2</sub> content whether the blood was centrifuged at 37°C or 5°C.

We could find no significant difference in the concentrations of Na, K, Mg, Ca, Cl, total CO<sub>2</sub> and protein whether the blood was centrifuged at 38°C or at room temperature (22-24°C) (table 7).

### Nitrogen factor

Originally we used a catalyst mixture containing selenium, but this leads to a loss of nitrogen (HILLER *et al* 1948, WILLITS *et al* 1949 1950). This became evident when with our original technique we determined the N-factor on dialysed alcohol-ether extracted bovine albumin and obtained a value of 6.60. Although most of the values published for the N-factor are well above 6.25 and SUNDERMAN *et al* (1958) – apparently using the same technique as Willits *et al* – have recently reported a value of about 6.55 for all electrophoretic fractions of serum protein, HILLER *et al* (1948) in a very careful investigation found a value of 6.22 and 6.24 for two different samples of pooled human plasma. Their macro-Kjeldahl results showed good agreement with the results obtained with the Dumas method, and their observations are so well documented that we accept their conclusion, that for plasma proteins the protein/N ratio is 6.25 after correction for non-protein nitrogen. With our present technique of Kjeldahl analysis we found a N factor of 6.32 for one sample of dialysed alcohol-ether extracted human albumin, and of 6.25 for one sample of human globulin treated in the same way. In view of the fact that in our experiments the fat extraction has been less rigorous than in those of Hiller *et al* the dry albumin may have included more lipids in our case. We conclude therefore that with our technique of N analysis the use of 6.25 as N-factor is warranted. This is supported by the fact that we found the partial specific volume for the albumin sample to be 0.738 and for the globulin sample 0.735 which agrees reasonably well with the results of the specialised measurements quoted by PHELPS & PUTNAM (1960), to wit 0.733 and 0.739 respectively.

### Protein (biuret method)

This method was used for the determination of protein in all our experiments.

### Technique

The analysis was done according to the description of GORNALL *et al* (1949). 1.5 g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 6.0 g  $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$  and 300 ml 2.5 N NaOH are dissolved in distilled water to a total volume of 1 litre. In a test tube 8 ml of this reagent are mixed with 1 ml of distilled water then 0.1 ml plasma is added and the contents of the tube thoroughly mixed again by swirling. The color is left to develop at room temperature for 60 minutes, when it has become practically constant (less than 1% further increase in optical density (O.D.) over the next 15 minutes). Then the transmittance is read at 540 m $\mu$  in an Evelyn colorimeter against a blank of 8 ml reagent and 2.1 ml distilled water. Determinations are done in duplicate and the same tube is used for all colorimeter readings. The transmittance is read to the nearest  $\frac{1}{2}$  scale unit. At 60 g/L protein this corresponds to 0.3 g/L, at 100 g/L protein to 0.7 g/L.

The trichloroacetic acid filtrate of fresh normal plasma was found to contain no biuret positive substances. The concentration of the plasma per se to the O.D. was tested with a mock biuret reagent which contained all constituents with the exception of  $\text{CuSO}_4$ . Otherwise the procedure was as described above. Clear to slightly opalescent plasma samples did not influence O.D., distinctly icteric plasma gave slight increase only but both haemolysis and lipemia had marked effect. As incubated already the few haemolytic samples in our experiments were rejected and since all *in vivo* ultrafiltration experiments are done at the fasting state some degree of lipemia as only observed in the plasma of three patients with nephrosis. In these instances the

From the difference between wet weight and dry weight and the known volume of the pipet the water content of the sample was calculated in g per litre plasma at room temperature

Samples were determined in triplicate and with each set of determinations a blank of distilled water was run also in triplicate. All weighings were done on a Mettler analytical balance type H 16 which can be read to the nearest 0.01 mg

#### *Systematic error*

A 24-hours stay at 108°C was found to be sufficient since in 30 experiments no further change in weight was observed when drying was continued up to 72 hours, or vacuum extraction over  $P_2O_5$  was carried out additionally

Furthermore the measured density of distilled water never differed by more than 0.8 g % from the density to be expected at the temperature of ambient air the average value of the latter being 21.3°C (range 15.8–26.7). For a total of 121 determinations the mean difference between the two was -0.25 % (extremes +0.5 to -0.8%), the measured density being slightly lower. The difference would be fully explained if the temperature of the samples was on the average 1°C higher than that of the ambient air. In view of the unavoidable touching of the pipet it is concluded that there is no evidence of a significant systematic error when samples of distilled water are dried

#### *Accidental error*

From the triplicate values obtained in all *in vivo* ultrafiltration experiments the standard deviation of a single determination of water content was calculated and the standard error of the mean. This was done separately for the samples containing respectively 950 to 930, 929 to 910 and 909 to 840 g water per liter plasma. From table 10 it can be seen that expressed in absolute values, the SE of the mean was the same for the three groups

#### *Protein (macro-Kjeldahl method)*

The macro-Kjeldahl method was used to calibrate the biuret method accepting the factor 6.25 for converting grams of nitrogen to grams of protein

#### *Technique*

This was essentially the one described by WILLITS *et al* (1949, 1950). To 0.5 ml sample in a 100 ml Kjeldahl flask is added 7 ml catalyst mixture (4 g HgO, 100 g  $Na_2SO_4$  and 200 ml 96%  $H_2SO_4$  made up to 700 ml with distilled water). Digestion is continued for 30 minutes after the content of the flask has become clear, boiling has to be vigorous. After transferring the digested sample to the Parnas-Wagner apparatus 20 ml 50% NaOH + 5 ml 8%  $Na_2S_2O_3$  is added and distillation started. The  $NH_3$  is received in 10 ml 0.075 N  $H_2SO_4$  to which a bromocresolgreen-methylred indicator is added and the excess  $H_2SO_4$  is titrated with 0.1000 N NaOH. With each series of plasma determinations a blank and a standard solution of  $(NH_4)_2SO_4$  are analysed. With this technique we constantly recovered 99–101% of the nitrogen both for  $(NH_4)_2SO_4$  and for tryptophan. Plasma was corrected for non-protein nitrogen and duplicate determinations did not differ by more than 0.5%.

original sample. The pipets used for making up the different dilutions were calibrated against each other so that the simulated concentrations could be calculated from the sample's original protein concentration to within 0.2 %. For all samples the protein concentrations so obtained were plotted against the optical densities measured.

The resulting scatterdiagram is shown in fig. 9 main graph. It can be seen that the deviation from linearity starts at a protein concentration of about 80 g/L and that the use of a linear calibration line would underestimate the protein concentration by about 1-1.5 % at 100 g/L and by about 3 % at 140 g/L. The line drawn through the experimental points in fig. 9 main graph, is the final calibration line from which the conversion table was derived.

For the precise construction of its linear trajectory the following procedure was adopted. The relation between O.D. and protein concentration was considered separately for the 19 series of dilutions, and for each point within a series the observed deviation from linearity was expressed as percentage of the (hypothetical) protein concentration which would have been obtained if the relation O.D.-protein concentration were linear throughout. Thus for each point shown in the scatterdiagram of fig. 9 its 'percentage deviation from linearity' was obtained. Next, the values on the O.D. scale higher than 0.200 were subdivided in units of 0.0554 (which is equivalent to 10 g/L protein on the linear part of the calibration line) and for all experimental points falling within one such 'unit' of O.D. the mean 'percentage deviation from linearity' was calculated and plotted against the mean of their optical densities. Through the points so obtained the line of best fit was drawn. The result is shown in the small graph occupying the lower right-hand corner of fig. 9 for each group the range found for the percentage deviation from linearity is represented by a horizontal bar and the number of experimental points indicated.

A further indication that the relation between O.D. and plasma concentrations higher than 75 g/L was not linear is the following observation. In 19 *in vivo* ultrafiltration experiments the protein concentration of the samples containing more than 80 g/L plasma was determined both in the (normal) final plasma dilution of 1:101 and in a final dilution of 1:201. Again it was found that for higher protein concentrations the relation between O.D. and protein concentration is not a linear one, and the deviation from linearity in the range 80-120 g protein/L plasma calculated from these experiments was in perfect agreement with that shown in fig. 9.

The deviation from linearity was exactly the same whether O.D. was measured in the Ewiva colorimeter or in the Unicam SP 900 spectrophotometer and whether plasma was analysed or pooled bovine albumin.

Observations made by MINE (1945) suggest that with the method of CORNALL *et al.* a relative shortage of copper ions occurs when analysing plasma containing more than 120 g protein per litre. Originally we rejected this explanation for the shape of our calibration line because in first series of experiments no effect was observed when copper content and tartrate concentration of the reagent mixture were increased. Later we learned that STRICKLAND *et al.* (1961) made a study of copper binding by proteins in alkaline solutions and that they observed a rectilinear relation between O.D. and serum protein concentration, provided that the amount of protein per mole of copper ion did not exceed 4000 grams. With the method of CORNALL *et al.* the concentrations in the final reagent mixture for copper sulfate, tartrate and sodiumhydroxide are 0.0048 M/L, 0.017 M/L and 0.60 M/L respectively. In theory therefore, the concentration of copper ions is sufficiently high to guarantee a calibration line which is rectilinear up to a concentration of 3 g protein per litre of the final solution, corresponding with 300 g protein per litre plasma. This is not so, and we have also found that increasing the copper concentration to 0.01 M/L in the final reagent mixture / the actual concentration used by Strickland *et al.* did restore the rectilinearity of the calibration line for the protein range shown in fig. 9. Changes in the concentration of tartrate or of sodiumhydroxide had no effect. Clearly therefore, the shape of our cal-



O D of the plasma itself was determined, and the optical density measured with the biuret reaction corrected accordingly

### Calibration line

The O D so obtained is directly converted to protein concentration with the use of a table which was constructed from the following data. Up to a protein concentration of 75 g/L the relation between O D and protein concentration was found to be linear and, from macro-Kjeldahl analyses on plasma containing 52 to 75 g/L protein, calculated as  $\text{protein (g/L)} = 181.0 \times \text{O D}$  with our technique. This relation was repeatedly determined during the course of our experiments and the conversion factor found varied from 179.0 to 182.0 (8 determinations). However for protein concentrations higher than 75 g/L the relation with O D becomes alinear the conversion factor increasing steadily. At a concentration of 100 g/L protein the deviation from linearity is only 1-1.5 %, which presumably explains why Gornall *et al* concluded that the relation was linear up to that point. But during *in vivo* ultrafiltration the protein concentration can rise to 120 g/L - with pathological plasma even to 160 g/L.

The relation between O D and protein concentrations higher than 75 g/L was therefore determined experimentally and the extended calibration line thus obtained used for the calculation of the corresponding part of the conversion table. For these experiments 19 samples were used: 1 sample of purified bovine serum albumin, 3 different

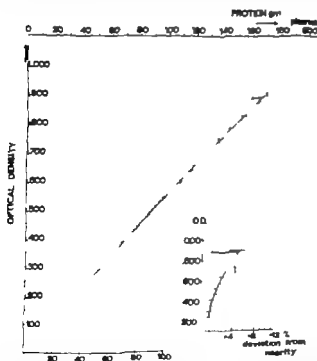


Fig. 9 Construction of calibration line used with the biuret method. See text for explanation

samples of individual plasma and 15 different samples of pooled plasma. Their protein concentrations ranged from 55 to 79 g/L and therefore fell within the linear part of the calibration line. By varying for each sample the ratio between plasma and distilled water while keeping constant final volume and the concentration of the biuret reagent we simulated protein concentrations ranging from 0.15 times to 3 times the value of the

original sample. The pipets used for making up the different dilutions were calibrated against each other so that the simulated concentrations could be calculated from the sample's original protein concentration to within 0.2%. For all samples the protein concentrations so obtained were plotted against the optical densities measured.

The resulting scatterdiagram is shown in fig. 9 main graph. It can be seen that the deviation from linearity starts at a protein concentration of about 80 g/L and that the use of a linear calibration line would underestimate the protein concentration by about 1-1.5% at 100 g/L and by about 3% at 140 g/L. The line drawn through the experimental points in fig. 9 main graph, is the final calibration line from which the conversion table was derived.

For the precise construction of its linear trajectory the following procedure was adopted. The relation between O.D. and protein concentration was considered separately for the 19 series of dilutions, and for each point within a series the observed deviation from linearity was expressed as percentage of the (hypothetical) protein concentration which would have been obtained if the relation O.D.-protein concentration were linear throughout. Thus for each point shown in the scatterdiagram of fig. 9 its 'percentage deviation from linearity' was obtained. Next, the values on the O.D. scale higher than 0.200 were subdivided in units of 0.0534 (which is equivalent to 10 g/L protein on the linear part of the calibration line) and for all experimental points falling within one such 'unit' of O.D. the mean percentage deviation from linearity was calculated and plotted against the mean of their optical densities. Through the points so obtained the line of best fit was drawn. The result is shown in the small graph occupying the lower right-hand corner of fig. 9 for each group the range found for the percentage deviation from linearity is represented by horizontal bar and the number of experimental points indicated.

A further indication that the relation between O.D. and plasma concentrations higher than 75 g/L was not linear is the following observation. In 19 *in vivo* ultrafiltration experiments the protein concentration of the samples containing more than 80 g/L plasma was determined both in the (normal) final plasma dilution of 1/101 and in a final dilution of 1/201. Again it was found that for higher protein concentrations the relation between O.D. and protein concentration is not a linear one, and the deviation from linearity in the range 80-120 g protein/L plasma calculated from these experiments was in perfect agreement with that shown in fig. 9.

The deviation from linearity was exactly the same whether O.D. was measured in the Eytex colorimeter or in the Unicam SP 500 spectrophotometer and whether plasma was analysed or purified bovine albumin.

Observations made by Miron (1945) suggest that with the method of Gornall *et al.* a relative shortage of copper ions occurs when analysing plasma containing more than 120 g protein per litre. Originally we rejected this explanation for the shape of our calibration line because in first series of experiments no effect was observed when copper content and tartaric concentration of the biuret reagent were increased. Later we learned that Strickland *et al.* (1961) made

a study of copper binding by proteins in alkaline solutions and that they observed a rectilinear relation between O.D. and serum protein concentration, provided that the amount of protein per mole of copper ion did not exceed 600 grams. With the method of Gornall *et al.* the concentrations in the final reagent mixture for copper sulfate, tartaric acid and sodiumhydroxide are 0.0048 M/L, 0.017 M/L and 0.60 M/L respectively. In theory therefore, the concentration of copper ions is sufficiently high to guarantee calibration linearity up to a concentration of 3 g protein per litre of the final solution, corresponding with 300 g protein per litre plasma. This is not so and we have since found that increasing the copper concentration to 0.01 M/L in the final reagent mixture (the actual concentration used by Strickland *et al.*) did restore the rectilinearity of the calibration line for the protein range shown in fig. 9. Changes in the concentration of tartaric acid or of sodiumhydroxide had no effect. Clearly therefore, the shape of our cal-

bration line is after all the result of too low a copper concentration. We have no explanation for the misleading results of our first series of experiments.

As a final test different dilutions of 4 plasma samples were analysed, both with the method of Gornall *et al* using the conversion table described above, and according to Stenckland *et al*. The values obtained agreed to within 0.5 % even though the protein concentration in the final reagent mixture was as high as 1.8 g/L (corresponding to 180 g protein per litre plasma).

#### *Systematic error as judged from comparison with macro-kjeldahl*

In table 11 the macro-Kjeldahl method is compared with the biuret method using the correct calibration line. The values agree to within 1 % over the range 60 to 111 g/L plasma protein with the notable exception of the two samples B1 and B2, and most of the plasma samples from patients with multiple myeloma.

We have no explanation for the discrepancy observed with B1 and B2, but the patients with multiple myeloma all had very high concentrations of myeloma protein and it is questionable whether the normal N-factor holds for these proteins. It is also possible that in these proteins the relation between N content and biuret reactive groups is different from that found for the ordinary plasma proteins (MEHL *et al* 1949) but Gornall *et al* could find no difference in the case of an abnormal serum globulin (precipitated with 13.5 %  $\text{Na}_2\text{SO}_4$  but not further specified).

It is concluded that at the protein concentrations observed in our experiments, no systematic discrepancy exists between the values obtained with the macro-Kjeldahl method and those obtained with the biuret method unless there is a high concentration of abnormal protein.

#### *Accidental error*

From all the duplicate values obtained in the *in vitro* ultrafiltration experiments the standard deviation of a single determination was calculated and the standard error of the mean. This was done separately for the protein concentration ranges shown in table 12. The higher the protein concentration the smaller the percentage error. Throughout the protein concentration range encountered in our experiments the SE of the mean (expressed in g per litre) remains the same.

#### *Protein fractions*

We have used both a salting-out technique and paper electrophoresis

#### *Salting-out*

The method of MAJOR (1946) was used, which discerns an albumin, a pseudo-globulin and an euglobulin fraction by differentiation of solubility curves, using a 28 and a 19 disodium sulphate solution respectively. The protein content of the fractions obtained is determined with the biuret method.

#### *Paper electrophoresis*

This was performed on Schleicher and Schüll paper 2043 B using a barbital buffer (pH 8.6) and a voltage of 110 V. After staining the proteins with amido-black 10 B the strips are cut, eluted with NaOH and the fractions determined quantitatively by colorimetry. Technique, accuracy and reproducibility of the method have been described in detail by ENNEKING (1956). However, as Enneking studied sera and we have analysed

plasma as well, some remarks must be added. When plasma is analysed by paper electrophoresis, one finds a band situated between the  $\beta$ - and the  $\gamma$ -bands, which is only very vaguely seen in serum obtained from the same blood. For a correct assessment of the protein composition of the plasma samples it is necessary to cut out this part of the strip separately and determine the fibrinogen content by elution, subsequent colorimetry of the eluate and calculation of 'fibrinogen' by the use of an appropriate factor necessary to correct for the difference in the adsorption of colour between albumin and fibrinogen.

**Correction factor for fibrinogen** This factor was not determined by Enneking but could be found in the following manner: a concentrated solution of fraction I (cold ethanol method 6), containing 77.4 g/L protein and a solution of crystalline human albumin containing 78.5 g/L protein, were compared by pipetting 0.1 ml of each solution on paper, colouring with amido-black, drying, elution and colorimetry. For the eluate of fraction I an O.D. of 297 was found, for the albumin eluate an O.D. of 427 and this gives a correction factor of  $(427/297)$   $(77.4/78.5) = 1.42$ . With paper electrophoresis the solution of fraction I was found to contain  $\gamma$ -globulin, 'fibrinogen' and small amounts of  $\beta$ - and  $\alpha$ -globulin. From the correction factor calculated for the solution as a whole and the correction factor found by Enneking for respectively the  $\gamma$ - (1.76),  $\beta$ - (1.40),  $\alpha_2$ - (1.50), and  $\alpha_1$ - (1.52) globulins, the correction factor for fibrinogen was finally calculated at 1.34 (the actual values found in three experiments were 1.30, 1.38 and 1.35). To check the correctness of these calculations a diluted solution of fraction I was added to a sample of normal plasma, and then the normal plasma, the diluted solution of fraction I and the mixture were all three analysed by electrophoresis using the above-mentioned correction factors. The result is given in table 13 and shows a reasonable recovery (106%) for 'fibrinogen' which is at least as good as, if not better than, the recovery found for the globulin fractions.

#### *Systematic error*

When comparing his method with the moving boundary electrophoresis, Majoor found the quantity of the albumin fraction practically equal with both methods. The  $\alpha$ -globulin fraction was quantitatively somewhat larger than the  $\gamma$ -globulin fraction (MAJOOR 1947). ENNEKING (1936) found (in serum of patients with renal disease) with paper electrophoresis the albumin fraction about 8% lower than with the method of Majoor and the  $\gamma$ -globulin fraction considerably lower than the  $\alpha$ -globulin fraction as determined with the method of Majoor.

It must be concluded that, quantitatively the albumin fraction determined according to Majoor is practically equal to the electrophoretic albumin fraction, but that the  $\alpha$ -globulin fraction contains not only  $\gamma$ -globulin, but also  $\beta$ -globulin and possibly  $\alpha$ -globulin. The amount of  $\beta$ - and  $\alpha$ -globulin precipitated as  $\alpha$ -globulin presumably depends on the relative concentrations of the fractions (ENNEKING 1956).

#### **Na and K**

These were determined by flame photometry

#### *Apparatus*

This was constructed by Dr VRIES and is described in GORTER & DE GRAAFF (1955). It operates with two barrier-layer cells and two galvanometers, so that sodium and

potassium determination can be done simultaneously. No internal lithium standard is used. The atomiser consists of two wide bore glass capillaries with drawn-out tip and mounted at right angles. Air is used at a pressure of 900 cm Hg which is kept constant within 0.4%. The atomiser consumes about 16 ml sample per minute. The burner provided with a fine mesh and gives a stable flame. Propane gas is used at a pressure of 45 cm H<sub>2</sub>O.

For the Na and K analysis a combination of Corning glass filters is used the characteristics of which are shown in fig. 10.



Fig. 10 - Transmittance of filters used in flame photometer

The galvanometers are mounted on a rigid support against the outer wall of the laboratory building. In the course of our studies the Na galvanometer had to be changed (at 10-12-60). The galvanometer scales were such that with the technique used and in the concentration range observed in our experiments, the smallest difference between two readings corresponded to a difference in Na concentration of respectively 0.25 mEq/L (first galvanometer) and 0.14 mEq/L (second galvanometer). The change of the galvanometer will be neglected in the presentation of our results since we found the same value for the SD of a single plasma Na determination before and after 10-12-60 and no difference in the experimental results was observed (see Addendum). For the K galvanometer the smallest difference between two readings corresponded to 0.02 mEq/L K.

The following substances were found to have no influence on the emission of an aqueous solution containing 140 mEq/L Na and 5 mEq/L K (all concentrations refer to the undiluted solutions): CaCl<sub>2</sub> (20 mEq/L), MgCl<sub>2</sub> (8 mEq/L), CuSO<sub>4</sub>, ZnSO<sub>4</sub>, MnCl<sub>2</sub>, Fe(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, CdSO<sub>4</sub>, ammoniummolybdate (each in a concentration of 0.5 mM/L), the anions SO<sub>4</sub> (145 mEq/L), HCO<sub>3</sub> (140 mEq/L) and PO<sub>4</sub> (100 mM/L) and the organic substances urea (6 g/L) and glucose (10 g/L). In confirmation of the literature H<sup>+</sup> ions were found to influence emission since a depression of the latter was observed when the pH value of the solution sprayed into the flame was below 6.0. In the dilution used by us the pH was higher than that, unless an acid protein precipitant had been used.

#### Technique

The initial stages of the procedure differed according to whether plasma (or serum) was analysed directly or after protein precipitation.

When plasma was not deproteinised we proceeded as follows. 0.5 ml plasma was injected with a calibrated syringe pipet into a 50 ml volumetric flask (Leerdam) and 1 : 100 diluted with electrolyte-free water.

When plasma was deproteinised the following procedure was used. To 0.5 ml plasma 10 ml 6% trichloroacetic acid was added. After centrifugation 8 ml supernatant was pipetted into a 40 ml volumetric flask (Leerdam) and electrolyte-free water added to the mark. In this case the final dilution of plasma was therefore 1 : 105.

In both cases the standard solutions were treated in the same way as the plasma samples.

Four standard solutions were used containing NaCl and KCl in the following concentrations (mEq/L): 120-3, 130-4, 140-5 and 150-6. With each series of determinations a blank (electrolyte-free water) and at least three of the standard solutions were run. After each sample the standard solution nearest in concentration was re-analysed. If the galvanometer reading for Na had changed, the sample and the standard solution were redetermined until identical readings were obtained for the standard solution before and after the analysis of the sample. Furthermore, when the difference between duplicate determinations was larger than 0.5 mEq/L for Na or 0.05 mEq/L for K, both solutions were re-analysed and the first pair of determinations discarded.

In the case of deproteinised plasma (or serum) the values obtained were always corrected for the error due to the volume change resulting from the removal of protein.

In plasma containing 70 g/L protein with a specific volume of 0.8, the protein occupies 5.6% of the plasma volume. In the procedure for deproteinisation one part of plasma is diluted with 20 parts of 6% trichloroacetic acid. In the final 1 : 21 dilution the protein occupies 0.26% of the total volume. As the protein is precipitated, only 99.74% of the original volume is left, which contains quantitatively the other solutes of the original plasma sample. Consequently their concentration decreases with 0.26%, whereas in the standard solutions, which do not contain protein, no relative increase in concentration of the solutes occurs. The necessary correction evidently depends on the degree of plasma dilution in the solution from which the protein is precipitated. For 1 : 6 dilution the correction is -0.9% for a 1 : 2 dilution -1.8%.

The values mentioned in the following pages for deproteinised plasma or serum always refer to corrected ones, the correction having been made from the known protein content of the sample and an assumed specific volume of 0.80.

In view of the discussion of systematic errors which is to follow it is necessary to mention already here that with the technique described above the SD for a single plasma determination was 0.1 mEq/L for Na and 0.01 mEq/L for K.

#### *Systematic error*

The fact that the values published for the normal plasma Na concentration after the introduction of the flame photometer still range from about 135 to about 145 mEq/L and the fact that no common operator can be detected to explain this variation (chapter II) lead one to suspect that systematic errors exist which differ according to the apparatus and technique employed. During the preparation of this manuscript Bergström and Holtman reported that they found, both for Na and K, a lower value when plasma was analysed than when the solution obtained after protein precipitation was analysed. They concluded that the latter value was correct and that the low value obtained with plasma was due to a 'protein interference effect' (see chapter II). We also found a difference between Na and K values obtained with plasma (or serum) and those obtained

potassium determination can be done simultaneously. No internal lithium standard is used. The atomizer consists of two wide-bore glass capillaries with drawn-out tip and mounted at right angles. Air is used at a pressure of 900 mm Hg which is kept constant within 25%. The burner consumes about 16 ml sample per minute. The burner is provided with a fine mesh and gives a stable flame. Propane gas is used at a pressure of 45 mm H<sub>2</sub>O.

For the Na and K analysis a combination of Corning glass filters is used, the characteristics of which are shown in fig. 10.



Fig. 10 - Transmittance of filters used in flame photometer

The photometers are mounted on a rigid support against the outer wall of the laboratory building. In the course of our studies the Na photometer had to be changed (at 0-12-57). The photometer scales were such that, with the technique used and in the concentrations to range observed in our experiments, the smallest difference between two readings corresponded to a difference in Na concentration of respectively 0.25 mEq/l (first photometer) and 0.14 mEq/l (second photometer). The error of the photometer will be neglected in the presentation of our results since we found the same value for the SD of a single plasma Na determination before and after 10-1-60, and no difference in the experimental results was observed (see Addendum). For the K photometer the smallest difference between two readings corresponded to 0.05 mEq/l K.

The following substances were found to have no influence on the emission of an aqueous solution containing 180 mEq/l Na and 5 mEq/l K (all concentrations refer to the undiluted samples): CaCl<sub>2</sub> (20 mEq/l), MgCl<sub>2</sub> (6 mEq/l), CaSO<sub>4</sub>, ZnSO<sub>4</sub>, MnCl<sub>2</sub>, Fe<sup>2+</sup>, H<sub>2</sub>SO<sub>4</sub>, CdSO<sub>4</sub>, ammoniummethylred (each in a concentration of 0.5 mM/l), the anions SO<sub>4</sub><sup>2-</sup> (145 mEq/l), HCO<sub>3</sub><sup>-</sup> (140 mEq/l) and PO<sub>4</sub><sup>3-</sup> (100 mM/l) and the organic substances urea (5 g/l) and glucose (10 g/l). In confirmation of the literature H ions were found to influence emission since a decrease of the latter was observed when the pH value of the solution sprayed into the flame was below 4.0. In the dilution used by us the pH was higher than that, unless an acid-trap was present had been used.

**Technique**  
The initial stages of the procedure differed according to whether plasma (or serum) was analysed directly or after protein precipitation.

When plasma was not deproteinised we proceeded as follows. 0.5 ml plasma was injected with a calibrated syringe-pipet into a 50 ml volumetric flask (Leerdam) and 1:100 diluted with electrolyte-free water.

When plasma was deproteinised the following procedure was used. To 0.5 ml plasma 10 ml 6% trichloroacetic acid was added. After centrifugation 8 ml supernatant was pipetted into a 40 ml volumetric flask (Leerdam) and electrolyte-free water added to the mark. In this case the final dilution of plasma was therefore 1:105.

In both cases the standard solutions were treated in the same way as the plasma samples.

Four standard solutions were used containing NaCl and KCl in the following concentrations (mEq/L): 120-3, 130-4, 140-5 and 150-6. With each series of determinations a blank (electrolyte-free water) and at least three of the standard solutions were run. After each sample the standard solution nearest in concentration was re-analysed. If the galvanometer reading for Na had changed, the sample and the standard solution were redetermined until identical readings were obtained for the standard solution before and after the analysis of the sample. Furthermore, when the difference between duplicate determinations was larger than 0.5 mEq/L for Na or 0.05 mEq/L for K, both solutions were re-analysed and the first pair of determinations discarded.

In the case of deproteinised plasma (or serum) the values obtained were always corrected for the error due to the volume change resulting from the removal of protein.

If plasma containing 70 g/L protein with a specific volume of 0.8, the protein occupies 5.6% of the plasma volume. In the procedure for deproteinisation one part of plasma is diluted with 20 parts of 6% trichloroacetic acid. In the final 1:21 dilution the protein occupies 0.26% of the total volume. As the protein is precipitated, only 99.74% of the original volume is left, which contains quantitatively the other solutes of the original plasma sample. Consequently their concentration increases with 0.26%, whereas in the standard solutions, which do not contain protein, no relative increase in concentration of the solutes occurs. The necessary correction evidently depends on the degree of plasma dilution in the solution from which the protein is precipitated. For 1:6 dilution the correction is -0.9% for Na and -2.8% for K.

The values mentioned in the following pages for deproteinised plasma or serum always refer to corrected ones, the correction having been made from the known protein content of the sample and an assumed specific volume of 0.90.

In view of the discussion of systematic errors which is to follow it is necessary to mention already here that with the technique described above the SD for a single plasma determination was 0.1 mEq/L for Na and 0.01 mEq/L for K.

#### *Systematic error*

The fact that the values published for the normal plasma Na concentration after the introduction of the flame photometer still range from about 135 to about 145 mEq/L and the fact that no common operator can be detected to explain this variation (chapter II) lead one to suspect that systematic errors exist which differ according to the apparatus and technique employed. During the preparation of this manuscript Bergström and Hultman reported that they found both for Na and K a lower value when plasma was analysed than when the solution obtained after protein precipitation was analysed. They concluded that the latter value was correct and that the low value obtained with plasma was due to a protein interference effect (see chapter II). We also found a difference between Na and K values obtained with plasma (or serum) and those obtained



potassium determination can be done simultaneously. No internal lithium standard is used. The atomiser consists of two wide bore glass capillaries with drawn-out tip and mounted at right angles. Air is used at a pressure of 90.0 cm Hg which is kept constant within 0.5 %. The atomiser consumes about 16 ml sample per minute. The burner is provided with a fine mesh and gives a stable flame. Propane gas is used at a pressure of 45 cm H<sub>2</sub>O.

For the Na and K analysis a combination of Corning glass filters is used the characteristics of which are shown in fig. 10.

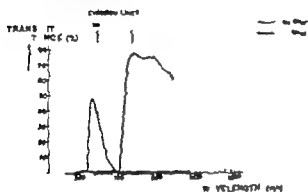


Fig. 10 - Transmittance of filters used in flame photometer

The galvanometers are mounted on a rigid support against the outer wall of the laboratory building. In the course of our studies the Na galvanometer had to be changed (at 10-12-60). The galvanometer scales were such that, with the technique used and in the concentration range observed in our experiments the smallest difference between two readings corresponded to a difference in Na concentration of respectively 0.25 mEq/L (first galvanometer) and 0.14 mEq/L (second galvanometer). The change of the galvanometer will be neglected in the presentation of our results since we found the same value for the SD of a single plasma Na determination before and after 10-12-60, and no difference in the experimental results was observed (see Addendum). For the K galvanometer the smallest difference between two readings corresponded to 0.02 mEq/L K.

The following substances were found to have no influence on the emission of an aqueous solution containing 140 mEq/L Na and 5 mEq/L K (all concentrations refer to the undiluted solutions): CaCl<sub>2</sub> (20 mEq/L), MgCl<sub>2</sub> (8 mEq/L), CuSO<sub>4</sub>, ZnSO<sub>4</sub>, MnCl<sub>2</sub>, Fe(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, CdSO<sub>4</sub>, ammoniummolybdate (each in a concentration of 0.5 mM/L), the anions SO<sub>4</sub> (145 mEq/L) and PO<sub>4</sub> (100 mM/L) and the organic substances urea (6 g/L) and glucose (10 g/L). In confirmation of the literature H<sup>+</sup> ions were found to influence emission since a depression of the latter was observed when the pH value of the solution sprayed into the flame was below 6.0. In the dilution used by us the pH was higher than that, unless an acid protein precipitant had been used.

#### Technique

The initial stages of the procedure differed according to whether plasma (or serum) was analysed directly or after protein precipitation.

When plasma was not deproteinised we proceeded as follows. 0.5 ml plasma was injected with a calibrated syringe-pipet into a 50 ml volumetric flask (Leerdam) and 1 : 100 diluted with electrolyte-free water.

When plasma was deproteinised the following procedure was used. To 0.5 ml plasma 10 ml 6% trichloroacetic acid was added. After centrifugation 8 ml supernatant was pipetted into a 40 ml volumetric flask (Leerdam) and electrolyte-free water added to the mark. In this case the final dilution of plasma was therefore 1 : 105.

In both cases the standard solutions were treated in the same way as the plasma samples.

Four standard solutions were used containing NaCl and KCl in the following concentrations (mEq/L): 120 - 3, 130 - 4, 140 - 5 and 150 - 6. With each series of determinations a blank (electrolyte-free water) and at least three of the standard solutions were run. After each sample the standard solution nearest in concentration was re-analysed. If the galvanometer reading for Na had changed, the sample and the standard solution were redetermined until identical readings were obtained for the standard solution before and after the analysis of the sample. Furthermore, when the difference between duplicate determinations was larger than 0.5 mEq/L for Na or 0.05 mEq/L for K, both solutions were re-analysed and the first pair of determinations discarded.

In the case of deproteinised plasma (or serum) the values obtained were always corrected for the error due to the volume change resulting from the removal of protein.

In plasma containing 70 g/L protein with a specific volume of 0.8, the protein occupies 5.6% of the plasma volume. In the procedure for deproteinisation one part of plasma is diluted with 20 parts of 6% trichloroacetic acid. In the final 1 : 21 dilution the protein occupies 0.26% of the total volume. As the protein is precipitated, only 99.74% of the original volume is left, which contains quantitatively the other solutes of the original plasma sample. Consequently their concentration increases with 0.26% hereinafter in the standard solutions, which do not contain protein, so relative increase in concentration of the solutes occurs. The necessary correction evidently depends on the degree of plasma dilution in the solution from which the protein is precipitated. For 1 : 6 dilution the correction is -0.9%, for 1 : 2 dilution -2.8%.

The values mentioned in the following pages for deproteinised plasma or serum always refer to corrected ones, the correction having been made from the above protein content of the sample and its assumed specific volume of 0.80.

In view of the discussion of systematic errors which is to follow it is necessary to mention already here that with the technique described above the SD for a single plasma determination was 0.1 mEq/L for Na and 0.01 mEq/L for K.

#### *Systematic error*

The fact that the values published for the normal plasma Na concentration after the introduction of the flame photometer still range from about 135 to about 145 mEq/L and the fact that no common operator can be detected to explain this variation (chapter II) lead one to suspect that systematic errors exist which differ according to the apparatus and technique employed. During the preparation of this manuscript Bergström and Hultman reported that they found, both for Na and K, a lower value when plasma was analysed than when the solution obtained after protein precipitation was analysed. They concluded that the latter value was correct and that the low value obtained with plasma was due to a protein interference effect (see chapter II). We also found a difference between Na and K values obtained with plasma (or serum) and those obtained

with deproteinised plasma (or serum) but from evidence to be presented below we must conclude that *with our technique and apparatus* for Na the values obtained with plasma or serum are nearly correct (0.1 mEq/L too low) whereas the values obtained after deproteinisation are on the average 2.5 mEq/L too high. For K these values are respectively 0.1 mEq/L too low and 0.1 mEq/L too high. To add a new term to the one coined by Bergström and Hultman *with our technique the protein interference effect is very small but the deproteinisation effect fairly large in the case of Na whereas the are of about equal magnitude in the case of K*

Apparently the protein interference effect and the deproteinisation effect vary for each apparatus and technique. At present we still have no satisfactory explanation for either of the two effects. These are being further studied in our department by DE VRIES and VAN DAATSELAAR and here we will only present the data from which we calculated the magnitude of these effects in our experiments

*Magnitude of the difference found between plasma (or serum) and deproteinised plasma (or serum) when using our technique* In 1958 DE VRIES and VAN DAATSELAAR observed that both the Na and the K concentration were found 1-3% lower when plasma (or serum) was analysed directly in the flame photometer than when it was analysed after deproteinisation (protein precipitated with 6% trichloroacetic acid 1:21 concentration corrected for volume change due to removal of protein) [unpublished results]

In our experiments we consistently found a difference between plasma and deproteinised plasma both for normal and for pathological plasma (or serum), including that obtained from patients with myeloma and severe uraemia. The difference was the same when protein precipitants other than trichloroacetic acid were used (HCl perchloric acid, acetic acid, uranylacetate) provided protein precipitation occurred at the same plasma dilution (i.e. 1:21)

The magnitude of the difference is demonstrated by the results obtained in the 19 *in vivo* ultrafiltration experiments in which Na and K were determined both in plasma and in deproteinised plasma (table 14). For Na there appears to be a positive correlation with the protein concentration of the plasma

*Evidence that with our technique the values obtained with plasma are very nearly correct* This evidence is mainly provided by experiments in which Na and K concentration were analysed in plasma before and after ashing, and by experiments in which electrolyte-free serum was added in increasing amounts to a fixed quantity of a standard solution containing NaCl and KCl. The series of ashing experiments to be reported did not include the ashing of deproteinised plasma, since at the time the ashing of the protein-free supernatant failed to give reproducible results presumably because of the large quantity of fluid which has to be ashed. This increases the chances that in the course of the ashing procedure losses occur as a result of splattering

(1) **ASHING EXPERIMENTS** - Fresh plasma samples with a protein concentration ranging from 50 to 160 g/L were analysed for Na and K, in a number of cases also the deproteinised plasma (6% trichloroacetic acid, plasma dilution 1:21). The plasma samples were then ashed, together with samples of standard solution and the Na and K content of the ash determined.

Seven crucibles containing 0.5 ml sample + 0.5 ml 4 N  $H_2SO_4$  were heated for at least 2 hours on a steam bath, dried in an electric stove at  $110^\circ C$  for one night and finally transferred to an electric furnace the temperature of which was gradually raised from  $200^\circ C$  to  $300^\circ C$ . As soon as their contents had become completely white (after 5 to 8 days), the crucibles were removed, the ash quantitatively dissolved in distilled water to a total volume of 30 ml, and the solution analysed for Na and K. As the standard solutions always gave the same galvanometer reading before and after the ashing, it would appear that no Na or K was lost. When in later experiments it was found that no loss of Na and K occurred at  $700^\circ C$ , the period in the furnace could be shortened to 4 hours at  $700^\circ C$ . The ash was found to contain sulphate, very small amount of phosphate, no chloride and no arsenicum.

The results are presented in table 15. It can be seen that in the case of Na the concentration found in plasma is on the average 0.1 mEq/L. lower than that found in the ash. The difference ranges from +0.8 to -1.0 mEq/L, and no correlation was found with the protein concentration of the sample. In the 12 instances in which deproteinised plasma (not ashed!) has also been analysed, the latter values were on the average 2.3 mEq/L. higher than the values obtained on plasma.

In the case of K the concentration in plasma is on the average 0.11 mEq/L. lower than in the ash, the difference ranging from +0.08 to -0.32. This covers about  $\frac{1}{2}$  of the difference observed between plasma and deproteinised plasma but - as in the case of Na - there appears to be no correlation with protein concentration.

(2) ADDITION OF ELECTROLYTE-FREE SERUM TO STANDARD SOLUTION - Serum was dialysed, for 24 hours at  $20^\circ C$  under sterile conditions in Viking cellophane bags, against distilled water. The pH of the latter was kept at about 5.0 by the addition of HCl. After dialysis the serum samples contained less than 0.5 mEq/L. (Na + K). Increasing amounts of the serum were then added to a fixed amount of standard solution and the effect on the flame photometer reading was determined.

Into 50 ml volumetric flasks 0.5 ml of standard solution, containing 140 mEq/L. NaCl and 5 mEq/L. KCl, was injected and respectively 0, 0.5, 1.0, 1.5 etc. ml of the dialysed serum added. One flask always contained 4 ml serum without NaCl solution in order to determine the amount of Na and K still present in the dialysed serum. The flasks were then filled to the 50 ml mark with distilled water and the contents analysed on the flame photometer, together with a set of standard solutions. The galvanometer readings obtained were expressed in terms of mEq/L., corrected for the amount of Na or K still present in the serum after dialysis, and then plotted against the protein concentration of the flasks contents, multiplied by 100. In this way protein concentration is expressed as if plasma samples with increasing protein concentration had been analysed in dilutions of 1/100.

From table 16 and fig. 11 it can be seen that protein concentrations higher than 30 g/L. have an increasingly depressive effect on the flame photometer reading, but in the range generally observed in the *in vivo* ultrafiltration experiments, i.e. 60 to 120 g/L., the magnitude of the depression remains small in the case of Na. In the plasma of patients with myeloma occasionally protein concentrations as high as 160 g/L. were observed and in that case a more severe depression might be expected to occur. The effect on K determination has been studied in one experiment only - as in the ashing experiment - it is evident that the percentage error is considerably larger than in the case of Na, but the same trend exists.

with deproteinised plasma (or serum) but from evidence to be presented below we must conclude that *with our technique and apparatus* for Na the values obtained with plasma or serum are nearly correct (0.1 mEq/L too low) whereas the values obtained after deproteinisation are on the average 2.5 mEq/L too high. For K these values are respectively 0.1 mEq/L too low and 0.1 mEq/L too high. To add a new term to the one coined by Bergström and Hultman *with our technique the protein interference effect is very small but the deproteinisation effect fairly large in the case of Na whereas they are of about equal magnitude in the case of K*.

Apparently the protein interference effect and the deproteinisation effect vary for each apparatus and technique. At present we still have no satisfactory explanation for either of the two effects. These are being further studied in our department by DE VRIES and VAN DAATSELAAR and here we will only present the data from which we calculated the magnitude of these effects in our experiments.

*Magnitude of the difference found between plasma (or serum) and deproteinised plasma (or serum) when using our technique.* In 1958 DE VRIES and VAN DAATSELAAR observed that both the Na and the K concentration were found 1-3% lower when plasma (or serum) was analysed directly in the flame photometer than when it was analysed after deproteinisation (protein precipitated with 6% trichloroacetic acid (1:21) concentration corrected for volume change due to removal of protein) [unpublished results].

In our experiments we consistently found a difference between plasma and deproteinised plasma both for normal and for pathological plasma (or serum) including that obtained from patients with myeloma and severe uraemia. The difference was the same when protein precipitants other than trichloroacetic acid were used (HCl perchloric acid, acetic acid, uranylacetate) provided protein precipitation occurred at the same plasma dilution (i.e. 1:21).

The magnitude of the difference is demonstrated by the results obtained in the 19 *in vivo* ultrafiltration experiments in which Na and K were determined both in plasma and in deproteinised plasma (table 14). For Na there appears to be a positive correlation with the protein concentration of the plasma.

*Evidence that with our technique the values obtained with plasma are very nearly correct.* This evidence is mainly provided by experiments in which Na and K concentration were analysed in plasma before and after ashing, and by experiments in which electrolyte free serum was added in increasing amounts to a fixed quantity of a standard solution containing NaCl and KCl. The series of ashing experiments to be reported did not include the ashing of deproteinised plasma, since at the time the ashing of the protein-free supernatant failed to give reproducible results, presumably because of the large quantity of fluid which has to be ashed. This increases the chances that in the course of the ashing procedure losses occur as a result of splattering.

(1) **ASHING EXPERIMENTS** - Fresh plasma samples with a protein concentration ranging from 50 to 200 g/L were analysed for Na and K, in a number of cases also the deproteinised plasma (6% trichloroacetic acid plasma dilution 1:21). The plasma samples were then ashed together with samples of standard solution and the Na and K content of the ash determined.

deproteinisation effect of the order of 2.5 mEq/L in the case of Na (provided the protein is precipitated by 6% trichloroacetic acid [21]).

*Systematic error of Na and K determination in plasma (or serum) in our experiments.* In the case of Na the ashing experiments indicate that the average error is 0.1 mEq/L, whereas from the results of the experiments in which electrolyte-free serum was added to standard solution the average error would appear to be 0.2 mEq/L at 70 g/L protein and 0.4 mEq/L at 120 g/L protein. This effect of an increase in protein concentration was not observed in the ashing experiments, where the accuracy is necessarily somewhat less. Table 16 moreover demonstrates that different plasma samples vary greatly in this respect. In view of the fact that considerably more samples were studied in the ashing experiments, the following conclusions appear reasonable.

On the average, Na concentration is underestimated by 0.1 mEq/L at a protein concentration of 60 g/L, and this error doubles when the protein concentration increases from 60 to 120 g/L. In the case of K the percentage error is considerably larger and from the experimental evidence presented we conclude that here also the underestimate is on the average 0.1 mEq/L at a protein concentration of 60 g/L, and doubles when the protein concentration increases from 60 to 120 g/L.

No attempt will be made to correct individual values for this error but it will be considered in the discussion of our results.

#### *Accidental error*

As mentioned before, the standard solution was redetermined after each sample, and the value obtained for the latter was only accepted if the standard readings before and after were identical. The gain in reproducibility is evident from table 17 which compares the results obtained by measuring 10 dilutions (1:100) of the same plasma in the manner described (A), with those obtained by simply determining the standard solution between samples and calculating the sample value by interpolation (B). The degree of reproducibility in series A (table 17) is of the same order as the SD of a single determination and the SE of the mean of duplicate determinations calculated from the 543 duplicate determinations obtained in the *in vivo* ultrafiltration experiments (table 18).

Since normally the duplicate determination is known as such, this could have influenced the reading of the duplicate, and the results of the duplicate determinations would then erroneously suggest a high reproducibility. The following experiment was therefore done. From two standard solutions, containing respectively 130.0 mEq/L NaCl + 4.0 mEq/L KCl and 140.0 mEq/L NaCl + 5.0 mEq/L KCl, the following mixtures were prepared with calibrated pipets: 10 + 0 ml, 9 + 1 ml, 8 + 2 ml, 0 + 10 ml. From the resulting solutions 1:100 dilutions were prepared in duplicate by the person who did the flame photometer determinations. Someone else then put the flasks in a random order unknown to the first person. After completing the determinations the values obtained were compared with the calculated values. Fig. 13 demonstrates that under these circumstances the reproducibility remained of the same order as that calculated from the duplicate determinations in the *in vivo* ultrafiltration experiments. For Na the difference between duplicate values was not more than 0.4 mEq/L and for K not more than 0.09 mEq/L. The mean values are close to the calculated values, with one notable exception. For the solution with a calculated concentration of 131.0 ± 4.1 a mean

The results of the ashing experiments and those obtained by adding serum to standard solution are in reasonable agreement for the range 50–120 g/L protein. It would appear that in normal plasma the depressive effect of protein on Na emission is almost negligible and not larger than in dialysed serum. However the relatively small difference between plasma and ashed plasma obtained in 4 hyperproteinaemic (and hypalbuminaemic) samples of the two patients with myeloma (Fr and Ja in table 15) suggests that

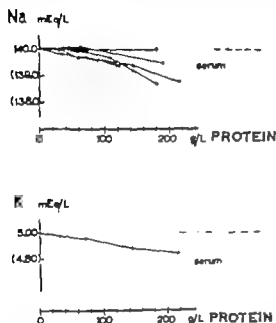


Fig. 11 Effect of adding increasing amounts of electrolyte-free serum to a NaCl and KCl containing standard solution (see table 15).

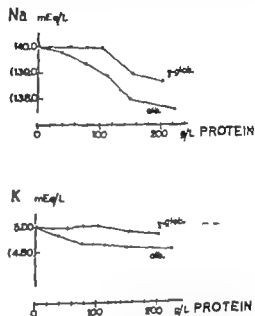


Fig. 12 Effect of adding increasing amounts of electrolyte-free albumin, respectively  $\gamma$ -globulin, to a NaCl and KCl containing standard solution.

the proteins differ in their depressive effect. We have therefore also done some experiments in which either albumin or  $\gamma$  globulin was added to a standard solution in the manner described above. The results are shown in fig 12. They indicate that the depression is more pronounced in the case of albumin than in the case of  $\gamma$ -globulin.

The effect of increasing protein concentration on the emission presumably is the result of a change in viscosity or surface tension. It is to be expected that the protein concentration at which the depressive effect becomes apparent will be different for different apparatus and will depend on the characteristics of the atomiser. But this explanation is not completely satisfactory since the effect is relatively larger for K than for Na.

After this chapter was written Van Daatselaar finally succeeded in ashing deproteinised plasma (6 / trichloroacetic acid 1 : 21) with the same accuracy as plasma. In preliminary experiments again practically no difference was observed between the Na concentration of plasma before and after ashing and again the Na concentration of the deproteinised plasma was found to be 2–4 mEq/L higher than that of plasma. But in addition it was now found that the Na concentration of the ashed deproteinised plasma was equal to the concentration found in plasma to within 0.1 mEq/L.

The latter experiments confirm our conclusion that when plasma (or serum) is analysed with our technique, the protein interference effect is of the order

deproteinisation effect of the order of 2.5 mEq/L in the case of Na (provided the protein is precipitated by 6% trichloroacetic acid [21]).

*Systematic error of Na and K determination in plasma (or serum) in our experiments.* In the case of Na the ashing experiments indicate that the average error is 0.1 mEq/L, whereas from the results of the experiments in which electrolyte-free serum was added to standard solution, the average error would appear to be 0.2 mEq/L at 70 g/L protein and 0.4 mEq/L at 120 g/L protein. This effect of an increase in protein concentration was not observed in the ashing experiments, where the accuracy is necessarily somewhat less. Table 16 moreover demonstrates that different plasma samples vary greatly in this respect. In view of the fact that considerably more samples were studied in the ashing experiments, the following conclusions appear reasonable.

On the average, Na concentration is underestimated by 0.1 mEq/L at a protein concentration of 60 g/L, and this error doubles when the protein concentration increases from 60 to 120 g/L. In the case of K the percentage error is considerably larger and from the experimental evidence presented we conclude that here also the underestimate is on the average 0.1 mEq/L at a protein concentration of 60 g/L, and doubles when the protein concentration increases from 60 to 120 g/L.

No attempt will be made to correct individual values for this error but it will be considered in the discussion of our results.

#### *Accidental error*

As mentioned before, the standard solution was redetermined after each sample, and the value obtained for the latter was only accepted if the standard readings before and after were identical. The gain in reproducibility is evident from table 17 which compares the results obtained by measuring 10 dilutions (1:100) of the same plasma in the manner described (A), with those obtained by simply determining the standard solution between samples and calculating the sample value by interpolation (B). The degree of reproducibility in series A (table 17) is of the same order as the SD of a single determination and the SE of the mean of duplicate determinations calculated from the 543 duplicate determinations obtained in the *in vivo* ultrafiltration experiments (table 18).

Since normally the duplicate determination is known as such, bias could have influenced the reading of the duplicate, and the results of the duplicate determinations would then erroneously suggest a high reproducibility. The following experiment was therefore done. From two standard solutions, containing respectively 130.0 mEq/L NaCl + 4.0 mEq/L KCl and 140.0 mEq/L NaCl + 5.0 mEq/L KCl, the following mixtures were prepared with calibrated pipets: 10 + 0 ml, 9 + 1 ml, 8 + 2 ml, 0 + 10 ml. From the resulting solutions 1:100 dilutions were prepared in duplicate by the person who did the flame photometer determinations. Someone else then put the flasks in a random order unknown to the first person. After completing the determinations the values obtained were compared with the calculated values. Fig. 13 demonstrates that under these circumstances the reproducibility remained of the same order as that calculated from the duplicate determinations in the *in vivo* ultrafiltration experiments. For Na the difference between duplicate values was not more than 0.4 mEq/L and for K, not more than 0.09 mEq/L. The mean values are close to the calculated values, with one notable exception. For the solution with a calculated concentration of  $131.0 \pm 4.1$  a mean



value of  $132.0 \pm 4.2$  was found, the duplicate values showing practically no difference. This value was consistently found on repeated determinations. It is therefore possible that the discrepancy is due to an error in the preparation of the solutions. This stresses the fact that in fig. 13 the results of the blind analysis are presented somewhat unfavourably because the error involved in making up the solutions was assumed to be zero whereas even with calibrated pipets an error of 0.1 / cannot be avoided.

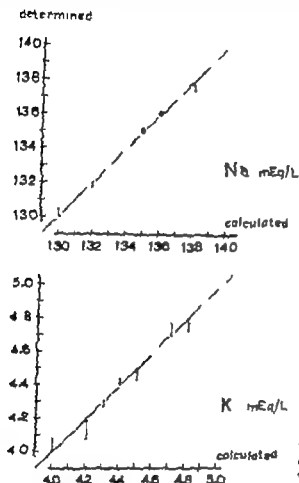


Fig 13 Values calculated compared with values determined in "blind" analysis of duplicate samples of extracted from two different NaCl + KCl solutions.

It is therefore concluded that the influence of bias on the results of Na and K analysis can be neglected.

#### Ca and Mg

These were determined by photoelectric colorimetric titration with EDTA (GORTER & DE GRASSIE 1955)

#### Technique

We used a micrometer buret, and magnetic stirring. The transmittance was read on a galvanometer to  $\frac{1}{2}$  scale unit. Titration proceeded as follows

(a) *Calcium* With a calibrated syringe-pipet 0.5 ml sample is injected into 6 ml 0.05 N NaOH with murexide as indicator. Then titration with 1.5 EDTA is started

transmittance being read at 565 m $\mu$ . Near the equivalence point EDTA is added in small amounts and the resulting transmittances are noted. This is continued until transmittance remains constant (the concentrations of the reagents are so chosen that the transmittance is 35-55 % at this point). Finally the amounts of EDTA successively added are plotted against the concomitant transmittances, and the equivalence point is determined by graphic interpolation.

(b) *Calcium + magnesium*. If the sumtitration immediately follows Ca titration, the pH of the titration milieu is first lowered by adding 0.1 ml 3 N  $\text{NH}_4\text{Cl}$ . Then eriochrom black is added as indicator and the wavelength changed to 660 m $\mu$ . If only a sumtitration is done 0.5 ml sample is added to 6 ml 0.05 N  $\text{NH}_4\text{OH}$  with eriochrom black. In both cases the further procedure is as described above.

With each series of analysis a reagent blank and a standard solution (5.0 mEq/L  $\text{CaCl}_2 + 2.0$  mEq/L  $\text{MgCl}_2$ ) are determined. Mg is obtained by subtraction. This is permitted since the same quantity of EDTA is needed to titrate 1 mEq of Ca and 1 mEq of Mg respectively.

#### *Systematic error*

When the sumtitration follows directly on Ca titration an error may be made: it was found that in the strongly alkaline milieu used for the Ca titration Mg is precipitated from a standard solution, but not from a protein containing solution. After the pH has been lowered by adding 3 N  $\text{NH}_4\text{Cl}$ , the finely dispersed precipitate redissolves in the course of 4 minutes. The sumtitration will therefore give erroneously low values for the standard solution if it is started immediately after adding the  $\text{NH}_4\text{Cl}$ . Since protein prevents the precipitation of Mg, no error occurs with plasma, and a 3-4 % overestimate of plasma Mg concentration is the result. However when sufficient time is allowed for Mg to redissolve, the titration of Ca + Mg following Ca titration gives the same value as the direct sumtitration.

Initially a solution containing 1.0 mEq/L  $\text{CaCl}_2 + 1.0$  mEq/L  $\text{MgCl}_2$  was added to one of the plasma samples at the end of each series of determinations. When it was found that recovery was always between 97 and 103 % of the calculated amount, this practice was abandoned. No difference was found between plasma and deproteinised plasma (6 % trichloroacetic acid 1:21) and even in haemolytic plasma no disturbing influence of Fe could be detected.

#### *Accidental error*

From the *in vitro* ultrafiltration experiments the SD of a single determination and the SE of the mean of a duplicate determination were calculated, both for Ca (509 duplicate determinations) and for the sumtitration of Ca and Mg (364 duplicate determinations) (table 19). For both determinations the absolute value of the SD was the same at high and at low concentrations.

#### *Cl*

This was determined by potentiometric titration with silvernitrate. The method is essentially that described by SANDERSON (1952), but a number of modifications have been made by Dr. VARRA.

### *Apparatus*

Instead of a buret a 2 ml glass syringe driven by a micrometer is used. The latter is graduated to 0.01 mm and has a length of 25 mm. To the tip of the syringe a plastic cannula is fitted with a silver wire projecting into its lumen (reference electrode). The measuring electrode is another silver wire which, with the tip of the cannula, reaches down into the flatbottom titration vessel ( $2 \times 5$  cm). The two silver wires are connected to a direct reading electronic millivoltmeter. A magnetic stirrer is used.

### *Technique*

After filling syringe and cannula with 0.5 N  $\text{AgNO}_3$  (care being taken that no air bubbles are trapped) the measuring electrode is rubbed with sandpaper and cleaned with distilled water. Then the titration vessel is filled with about 7 ml 0.2 N  $\text{H}_2\text{SO}_4$  in 0.2% chloride free gelatin (the gelatin was added to prevent precipitation of  $\text{AgCl}$  on the measuring electrode). Finally 0.5 ml sample is added from a calibrated syringe-pipet. After lowering the measuring electrode and cannula tip into the titration vessel, the voltmeter is adjusted for sensitivity and zero and the titration is started. When nearing the equivalence point,  $\text{AgNO}_3$  is added in very small amounts and sufficient time is allowed for the meter to adjust itself. In accordance with the experience of Sanderson it was found that the end point is very constant and occurs at the same potential in standard solutions and in plasma. Although for the latter the slope of the titration curve is slightly less steep it is our experience that the end point can be read without difficulty. For that reason we no longer make a graph of every titration, but titrate to the known potential of the equivalence point. With every series of analyses titrations were done on the  $\text{H}_2\text{SO}_4$ -gelatin solution (serving as a blank) and on two of the three available standard solutions which contained 100.0, 123.0 and 134.0 mEq/L of Cl respectively. The latter two were the  $120.0 \pm 3.0$  and  $130.0 \pm 4.0$  standard solutions used with the Na and K determinations. During a series of determinations one of the standard solutions was measured repeatedly in order to detect alterations in the electrode sensitivity. When the difference between duplicates was more than 1.0 mEq/L new duplicate determinations were done and the first pair was discarded.

### *Systematic error*

The presence of protein had no effect on the titration results. The addition of increasing amounts of electrolyte free plasma to 0.5 ml NaCl standard solution had no influence on the Cl concentration measured (table 20) and the Cl concentration measured in deproteinised plasma (6% trichloroacetic acid 1:11) did not differ significantly from that measured in plasma (table 21). Furthermore the use of the same standard solutions for Na and K analysis and for Cl analysis excludes one source of systematic error in the calculation of the difference between cation and anion concentrations.

In this connection the following experiment is of interest. Three polyethylene containers (used in the dialysis experiments to be mentioned in chapter V) and one glass container were filled with a NaCl solution and left to stand at  $38^\circ\text{C}$  for 40 hours. During this period several samples were taken from each container and analysed for Na and for Cl, the latter both with the potentiometric titration and with the method of Volhard as described in GORTER & DE GRAAFF (1955). The experiment was devised to see whether during dialysis experiments Na or Cl were released from, or absorbed by, the wall of the containers. The results of the experiments are shown in table 22. It can be seen that the Na determination and the potentiometric Cl determination give nearly

identical results and that their reproducibility is of the same magnitude. In contrast, the Cl<sup>-</sup> values obtained with the Volhard method are less reproducible and on the average significantly lower than the Na<sup>+</sup> concentrations, although the same standard solutions were used for all three methods.

#### *Accidental error*

The SD of a single determination, calculated from the 556 duplicate determinations done in the 100 mEq/L ultrafiltration experiments, was 0.11 mEq/L, and the SE of the mean 0.08 mEq/L (Cl<sup>-</sup> concentrations ranging from 95 to 106 mEq/L plasma).

determined

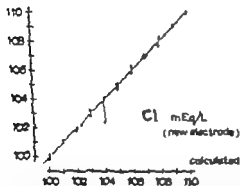
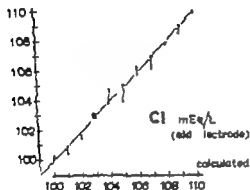


Fig. 14 Values calculated compared with values determined in "blind" analysis of duplicate samples of mixtures from two different NaCl solutions

Solutions with increasing Cl<sup>-</sup> concentrations were prepared from two standard solutions, containing respectively 100.0 mEq/L and 110 mEq/L NaCl. The samples obtained were analysed "blind" after the manner described for Na and K. Fig. 14 represents the result of the two experiments done. In one the measuring electrode was rather aged in the other experiment it was new. With the new electrode the standard deviation of single determination did not differ significantly from that calculated above. The only value which differed more than 0.3 mEq/L from the calculated value was indeed due to an error in determination on redetermination a value of 104.5 instead of 102.4 was found. In the experiment with the old electrode the standard deviation was about

### Apparatus

Instead of a buret a 2 ml glass syringe driven by a micrometer is used. The latter is graduated to 0.01 mm and has a length of 25 mm. To the tip of the syringe a plastic cannula is fitted with a silver wire projecting into its lumen (reference electrode). The measuring electrode is another silver wire which, with the tip of the cannula, reaches down into the flatbottom titration vessel (2 x 5 cm). The two silver wires are connected to a direct reading electronic millivoltmeter. A magnetic stirrer is used.

### Technique

After filling syringe and cannula with 0.5 N  $\text{AgNO}_3$  (care being taken that no air bubbles are trapped) the measuring electrode is rubbed with sandpaper and cleaned with distilled water. Then the titration vessel is filled with about 7 ml 0.2 N  $\text{H}_2\text{SO}_4$  in 0.2 chloride-free gelatin (the gelatin was added to prevent precipitation of  $\text{AgCl}$  on the measuring electrode). Finally 0.5 ml sample is added from a calibrated syringe-pipet. After lowering the measuring electrode and cannula up into the titration vessel, the voltmeter is adjusted for sensitivity and zero and the titration is started. When nearing the equivalence point,  $\text{AgNO}_3$  is added in very small amounts and sufficient time is allowed for the meter to adjust itself. In accordance with the experience of Sanderson it was found that the end point is very constant and occurs at the same potential in standard solutions and in plasma. Although for the latter the slope of the titration curve is slightly less steep it is our experience that the end point can be read without difficulty. For that reason we no longer make a graph of every titration but titrate to the known potential of the equivalence point. With every series of analyses titrations were done on the  $\text{H}_2\text{SO}_4$ -gelatin solution (serving as a blank) and on two of the three available standard solutions which contained 100.0, 123.0 and 134.0 mEq/L of Cl respectively. The latter two were the 120.0 + 3.0 and 130.0 + 4.0 standard solutions used with the Na and K determinations. During a series of determinations one of the standard solutions was measured repeatedly in order to detect alterations in the electrode sensitivity. When the difference between duplicates was more than 1.0 mEq/L new duplicate determinations were done and the first pair was discarded.

### Systematic error

The presence of protein had no effect on the titration results. The addition of increasing amounts of electrolyte free plasma to 0.5 ml NaCl standard solution had no influence on the Cl concentration measured (table 20) and the Cl concentration measured in deproteinised plasma (6% trichloroacetic acid 1:11) did not differ significantly from that measured in plasma (table 21). Furthermore the use of the same standard solutions for Na and K analysis and for Cl analysis excludes one source of systematic error in the calculation of the difference between cation and anion concentrations.

In this connection the following experiment is of interest. Three polyethylene containers (used in the dialysis experiments to be mentioned in chapter V) and one glass container were filled with a NaCl solution and left to stand at 38°C for 40 hours. During this period several samples were taken from each container and analysed for Na and for Cl, the latter both with the potentiometric titration and with the method of Volhard as described in GORTER & DE ORAAT (1955). The experiment was devised to see whether during dialysis experiments Na or Cl were released from, or absorbed by, the wall of the containers. The results of the experiments are shown in table 22. It can be seen that the Na determination and the potentiometric Cl determination give nearly

identical results and that their reproducibility is of the same magnitude. In contrast, the Cl values obtained with the Volhard method are less reproducible and on the average significantly lower than the Na concentrations, although the same standard solutions were used for all three methods.

#### Accidental error

The SD of a single determination, calculated from the 556 duplicate determinations done in the *in vivo* ultrafiltration experiments, was 0.11 mEq/L, and the SE of the mean 0.08 mEq/L (Cl concentrations ranging from 95 to 106 mEq/L plasma).

determined

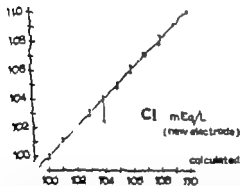
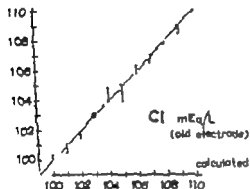


Fig 14 Values calculated compared with values determined in "blind" analysis of duplicate samples of standard from two different NaCl solutions

Solutions with increasing Cl concentrations were prepared from two standard solution containing respectively 100.0 mEq/L and 110 mEq/L NaCl. The samples obtained were analysed "blind" after the manner described for Na and K. Fig. 14 represents the results of the two experiments done. In one the measuring electrode was rather aged, in the other experiment it was new. With the new electrode the standard deviation of a single determination did not differ significantly from that calculated above. The only value which differed more than 0.3 mEq from the calculated value was indeed due to an error in determination on redetermination a value of 104.5 instead of 102.4 was found. In the experiment with the old electrode the standard deviation was about

four times as large (SD 0.40 mEq/L) but on the average the difference between the calculated and the measured concentration was less than 0.1 mEq/L.

Although there is therefore no evidence of bias, the difference in the results obtained with the two electrodes indicates that the reproducibility of the Cl determination must have varied in the one and a half year during which our experiments were done. However the policy of redetermining duplicate samples when they differed by 1 mEq/L or more,

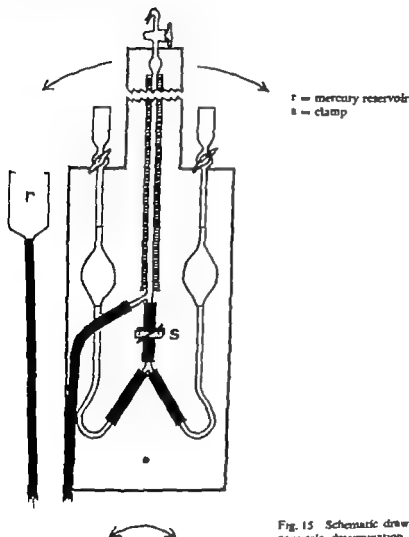


Fig. 15 Schematic drawing of apparatus used for manometric determination of CO content

should have had a stabilising influence. For that reason and because all our measurements were done with a new or fairly new electrode we accept the SE of the mean of a duplicate determination as calculated from the *in vivo* ultrafiltration experiments (i.e. 0.08 mEq/L).

#### Total CO<sub>2</sub>

This was determined by the manometric method of VAN SLYKE & NEILL (1924)

### Apparatus

We used the apparatus devised by Borst for gasometric urea determination. Its essentials are described by Borst (1942) and a schematic presentation is given in fig. 15

It consists of 2 extraction chambers with manometer tube in between. The glass parts are connected by thick-walled rubber tubing and the whole is mounted on a vertical wooden board and rigidly fixed. The board is made to shake in vertical plane by means of motor with eccentric transmission. The lower end of the extraction chamber units has the shape of an inverted question mark, so that air leaking through the connection with the manometer tube enters the latter and can be expelled before reading the gas pressure, by temporarily closing screw and raising the mercury reservoir. The manometer tube thus serves as an air exit, which arrangement limits the total number of stopcocks to three. Before reading gas pressure, the manometer is placed exactly vertical with the aid of levelling device mounted on the board.

The advantages of the apparatus are that duplicate determinations can be done simultaneously and that errors due to leakage are largely prevented.

### Technique

0.2 ml samples are used and injected with a calibrated syringe-pipet. With our apparatus one minute shaking at 200 revolutions/min. was found amply sufficient for complete extraction. The height of the mercury column is read to the nearest 0.5 mm. Otherwise technique, reagents, storage of reagents and correction factors are those described by Van Slyke and Neill (see also PETERS & VAN SLYKE 1932).

With each series of analyses a standard solution containing 25 mM/L  $\text{Na}_2\text{CO}_3$  is determined. It is prepared by dissolving 1.325 g dry  $\text{Na}_2\text{CO}_3$  (analytical grade) in distilled  $\text{CO}_2$ -free water to a total volume of 500 ml, and the concentration checked by titration with 0.1000 N HCl standard solution. The carbonate solution is stored in a closed pyrex bottle and a fresh solution is made every fortnight.

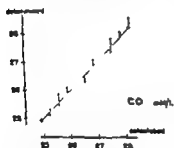


Fig. 14 Values calculated compared with values determined in "blind" analysis of duplicate samples of standards from two different  $\text{Na}_2\text{CO}_3$  solutions.

### Systematic error

For this the reader is referred to PETERS & VAN SLYKE (1932). It would appear that no systematic error arises if sufficient attention is given to the details of the technique as described by Van Slyke and Neill.

### Accidental error

From the 617 duplicate analyses performed in the *in vivo* ultrafiltration experiments the SD of single determination was calculated at 0.20 mM/L, and the SE of the mean



four times as large (SD 0.40 mEq/L) but on the average the difference between the calculated and the measured concentration was less than 0.1 mEq/L.

Although there is therefore no evidence of bias the difference in the results obtained with the two electrodes indicates that the reproducibility of the Cl determination must have varied in the one and a half year during which our experiments were done. However the policy of redetermining duplicate samples when they differed by 1 mEq/L or more,

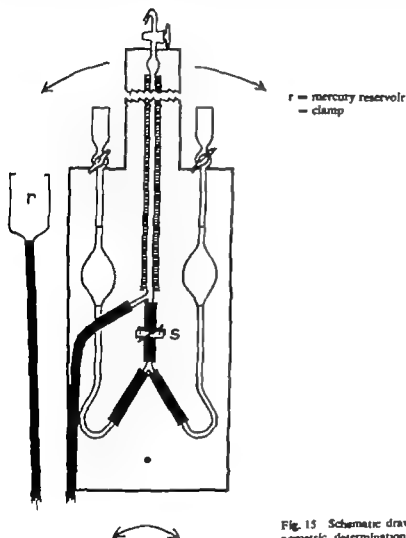


Fig. 15 Schematic drawing of apparatus used for manometric determination of CO content

should have had a stabilising influence. For that reason and because all our measurements were done with a new or fairly new electrode, we accept the SE of the mean of a duplicate determination as calculated from the *in vivo* ultrafiltration experiments (i.e. 0.08 mEq/L).

#### Total CO<sub>2</sub>

This was determined by the manometric method of VAN SLIKE & NEILL (1924)

The buffers were stored in closed pyrex glass containers at 20°C. Just prior to a series of determinations a small amount was decanted in stoppered glass tubes to be used for that day only. Fresh buffer solutions were prepared every two weeks. The difference between the 7.100 and the 7.400 buffer solutions was always  $0.300 \pm 0.003$  pH units.

#### Technique

After flushing the glass electrode several times with the sample fluid, equilibrium is generally reached within half a minute. The potential was read for four consecutive minutes and the difference between the last two readings was not more than 0.003 pH units unless the glass electrode had become too old. After each measurement the electrode was flushed with a 0.15 molar NaCl solution. At the conclusion of a series of determinations the electrode was stored with 7.400 buffer solution. A series of determinations was always started by measuring both buffer solutions repeatedly and alternately the buffer nearest to the pH of the samples was remeasured after every 2 to 3 samples.

All determinations were done in duplicate, and in our experiments the difference did not exceed 0.005 pH unit. In the *in vivo* ultrafiltration and the dialysis experiments the pH values were rounded off to the nearest 0.01 pH unit. This was thought to be sufficiently accurate for the purpose of the present investigation.

Finally to obtain the pH values for "true plasma," 0.01 was added to the blood pH values as a correction for the suspension effect of erythrocytes on the glass electrode (SEVERINGHAUS *et al.* 1956<sup>1</sup>). The pH values so obtained were used in the calculation of plasma bicarbonate.

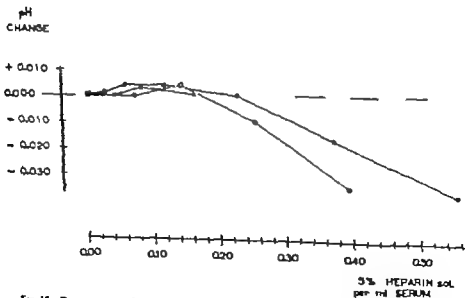


Fig. 17 Determination of pH. Effect of adding undiluted 5% heparin solution to serum (3 samples).

at 0.14 mM/L. As with Na, K and Cl the influence of bias on the duplicate results was tested by blind determination of a series of duplicate mixtures of a 24.9 mM/L and a 28.2 mM/L  $\text{Na}_2\text{CO}_3$  standard solution. The results are shown in fig. 16 and indicate that bias is not likely to have had a significant influence on our results.

## pH

### *Handling and storing of samples*

In the *in vivo* ultrafiltration experiments 3 to 5 ml blood was drawn anaerobically in a 5 ml Luer Lok syringe. The dead space of the syringes varied from 0.110 to 0.125 ml and was filled beforehand with 5% heparin solution. The capped syringe was immediately placed in ice water.

We have used the 5% heparin solution because with more diluted solutions clotting occasionally occurred in the concentrated blood obtained during *in vivo* ultrafiltration.

All measurements were done on whole blood and within 2 hours of sampling, after vigorous swirling of the syringe to resuspend the erythrocytes. We repeatedly measured the decrease in pH occurring during storage in ice water and always found it less than 0.010 after 2 hours.

As the measurement of all samples of one *in vivo* ultrafiltration experiment took up to 100 minutes, the first sample was, as a rule, redetermined at the end of the series. The decrease in pH was never more than 0.005 pH unit.

In the dialysis experiments the samples were also drawn anaerobically but they were stored at room temperature for up to 3 hours. No change in pH was observed in this period.

### *Apparatus and buffer solutions*

The pH was measured with the thermostatted micro glass electrode (G 297/G) and the saturated KCl-calomel electrode (K 497) of Radiometer using the A-C operated direct reading pH meter type PHM 22 with external meter of the same firm (reading precision 0.002 pH units). The two electrodes are connected by a free diffusion junction which is also thermostatted. The temperature inside the water jacket of the micro electrode was kept at 37.5°C ( $\pm 0.1^\circ$ ). For a detailed description of the electrode chain the reader is referred to SIGGAARD ANDERSEN *et al* (1960).

Two phosphate buffers were used. To the first a pH value of 7.100 was assigned (recipe:  $\text{KH}_2\text{PO}_4$  1.450 g,  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  4.030 g, distilled water to 500 ml). The second was considered to have a pH value of 7.400 (recipe:  $\text{KH}_2\text{PO}_4$  0.8487 g,  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  4.830 g, distilled water to 500 ml). The recipes were taken from VAN SLYKE *et al* (1949), but slightly modified in that in our case the final concentration of both buffer solutions was 0.0668 M/L instead of 0.0640 M/L.

The slight increase in concentration was found to result in lowering the pH by approximately 0.003. The pH values assigned to the 0.064 molar phosphate buffers by VAN SLYKE *et al* (1949) are of the Mac Innes convention, which defines the pH scale 0.015 unit lower than the National Bureau of Standards (BATES 1954). Consequently we measured at 37.5°C a pH value of 6.825 for the 0.025 equimolar phosphate buffer which, at a temperature of 34°C, has been accorded a pH value of 6.840 in the NBS scale (BATES 1954).

## HCO<sub>2</sub>

This was calculated from total CO<sub>2</sub> content and pH using the following equation (PETERS & VAN SLYKE 1932)

$$[\text{HCO}_2] = [\text{total CO}_2] \times \frac{1}{1 + \text{antilog}(\text{pK}_1 - \text{pH})}$$

The value 6.10 was accepted for pK<sub>1</sub> in all *in vivo* ultrafiltration experiments and in the dialysis experiments in which the pH was higher than 7.10. If it was lower pK<sub>1</sub> was corrected for the influence of pH using the values given by SEVERINGHAUS *et al.* (1956<sup>2</sup>).

The pK in the above value is in our case in reality pK' (see SØGAARD ANDERSEN 1962, 1963) for which the value of 6.10 seems the best approximation at 38°C and pH 7.40 (NBS scale). Since our pH scale is about 0.015 lower we presumably make a negative error in the calculation of HCO<sub>2</sub> by inserting the value of 6.10 for pK<sub>1</sub>. However, in normal plasma pH and CO<sub>2</sub> content this error would make an underestimate HCO<sub>2</sub> by less than 0.05 mEq/L and it can therefore be neglected in the *in vivo* ultrafiltration experiments. In the dialysis experiments where pH values lower than 6.90 were observed, the error must have been larger, e.g. about 0.08 mEq/L at pH 6.90 and about 0.1 mEq/L at pH 6.33 (which was the lowest pH value obtained). Since a systematic error in pH or in pK will affect the calculation of the HCO<sub>2</sub> concentration most at low pH values, we have corrected pK for pH influence in the experiments in which the pH value was below 7.10.

In the *in vivo* ultrafiltration experiments initially both total CO<sub>2</sub> and pH were determined for each sample, but later when the trend of pH during venous congestion had been established pH was only determined for every other sample. Thus it was necessary to calculate the pH of the intermediate samples by interpolation but since in the *in vivo* ultrafiltration experiments the difference in pH was never more than 0.02 pH unit for two consecutive samples, the calculated pH must have been correct to within  $\pm 0.01$  pH unit. In the dialysis experiments the pH was determined for every sample.

### Systematic error

The above mentioned equation is a restatement of the formula of Henderson-Hasselbalch in terms of total CO<sub>2</sub>, HCO<sub>2</sub>, pK<sub>1</sub> and pH. It is based on the assumption that total CO<sub>2</sub> is made up by HCO<sub>2</sub>, H<sub>2</sub>CO<sub>3</sub> and physically dissolved CO<sub>2</sub>. If CO<sub>2</sub> is present in another chemical combination this would cause an error in the calculation of HCO<sub>2</sub>. Two possible substances must be considered.

(a) *Carbonate*. In the physiological pH range the amount of carbonate present is very small. At pH 7.80, 38°C and a HCO<sub>2</sub> concentration of 30 mM/L, it can be estimated at 0.3 mM/L in a NaCl + NaHCO<sub>2</sub> solution with ionic strength 0.15. If we accept  $K_2 = 10^{-10}$  (HASTINGS & SENDROY 1925). Neglect of the presence of carbonate will therefore cause the contribution of CO<sub>2</sub> derivatives to the anionic valencies to be underestimated by 0.3 mEq/L when the pH of the plasma is 7.80. At pH 7.40 the underestimate will be about 0.1 mEq/L and at pH values below 7.00 it will be less than 0.05 mEq/L.

(b) *Carbamate*. It has been shown that in solutions containing amino acids or protein CO can react directly with —NH<sub>2</sub> groups to form a carbamino-compound. The reaction can be written as  $\text{RNH}_2 + \text{CO}_2 \rightleftharpoons \text{RNHCOO}^- + \text{H}^+$ . As —NH<sub>2</sub> does not react with CO<sub>2</sub>, carbamate formation occurs only at pH values higher than 6.0, and increases with increasing pH (FAURHOLT 1925, STADIE & O'BRIEN 1936). The reaction has been studied extensively in the case of Hb and HbO<sub>2</sub>, which proteins show a rather large

### *Systematic error*

(a) EFFECT OF HEPARIN ADDITION - The heparin solution used (Thromboliquine Organon) contains per ml circa 50 mg neutralised sodium heparinate. We found its pH about 6.8 (at 38°C) and the addition of increasing amounts of the heparin solution to serum did not affect the pH of the latter significantly unless more than 0.2 ml was added per ml serum. Fig. 17 indicates that at the ratio used in the *in vivo* ultrafiltration experiments (i.e. about 0.03-0.05 ml heparin solution per ml plasma) there should be no decrease in pH possibly a slight increase (less than 0.004).

Additional experiments were done with whole blood

Ten ml was drawn in a syringe containing 0.02 ml undiluted Thromboliquine, which was mixed with the blood by means of the air bubble contained in the dead space of the syringe. After expelling the air the capped syringe was put in ice water. A 5 ml Luer Lok syringe, its dead space filled with undiluted Thromboliquine, was also placed in ice water. After about 10 minutes 4 ml blood was transferred anaerobically from the 10 ml syringe into the 5 ml syringe, and both again placed in ice water. The pH of their contents was then measured alternately for about an hour the syringes being kept in ice water between measurements.

Two experiments were done in which no clotting of the blood occurred. In the first experiment the average pH values were 7.312 and 7.311 in the second experiment 7.371 and 7.370 (the first value refers to the 10 ml syringe). No significant difference in pH was therefore observed although the 10 ml syringe contained 0.002 ml Thromboliquine/ml blood and the 5 ml syringe 0.032 ml Thromboliquine/ml blood. We conclude that no systematic error in pH measurements resulted from the use of the undiluted heparin solution.

(b) STORAGE IN ICE WATER - As a result the pH values obtained in the *in vivo* ultrafiltration experiments may have been somewhat too low (between 0.000 and 0.008 pH unit).

(c) CORRECTION FOR SUSPENSION EFFECT OF ERYTHROCYTES - We applied an average correction based solely on the experiments of Severinghaus *et al*. We do not know whether for pathological blood the magnitude of the correction should be different.

(d) pH SCALE - Our pH values are based on the Mac Innes scale since this was apparently the one which Van Slyke *et al* used in their experiments. To convert our values to the NBS scale 0.015 should be added.

(e) TEMPERATURE OF BLOOD AT THE MOMENT OF SAMPLING - The pH measurements have been done at 37.5°C. If the temperature of the venous blood is in reality lower a systematic error is introduced. This error will be considered in chapter IV.

### *Accidental error*

As the difference between duplicate determinations was never more than 0.005 the SE of the mean of duplicate determinations was less than that

be calculated that an error of 0.02 in pH (at pH 7.30) will result in an error of 0.2 / in the calculation of bicarbonate concentration. Since in part of the *in vivo* ultrafiltration experiments about 50 / of the pH values have been calculated by interpolation, with a possible error of  $\pm 0.01$  pH unit, the SE of the mean of a duplicate determination presumably is somewhat larger for  $\text{HCO}_3^-$  than for total  $\text{CO}_2$ , but it seems unlikely that it is more than 0.20 mEq/L. At pH values below 7.00 however the spread must have been somewhat larger.

#### Partial carbon dioxide tension ( $\text{P}_{\text{CO}_2}$ ) in plasma

As we were only interested in the magnitude of the relative changes observed during *in vivo* ultrafiltration we thought it sufficient to estimate  $\text{P}_{\text{CO}_2}$  from total  $\text{CO}_2$  content and pH by means of the line chart published by VAN SLIKE & SENDROY (1928). The original graph was used as we learned from MÖLLER (1959) that the reproduction of the graph in PETERS & VAN SLIKE (1932) is erroneous.

#### Inorganic phosphate

This was determined as described by DE VRIES in GORTER & DE GRAAFF (1955). After deproteinisation, complex-ion formation with molybdic acid in the presence of ferrous ions is measured colorimetrically at 660–720 m $\mu$ , and compared with standard solutions (2.5 mg / and 5.0 mg / P). The maximum difference between duplicate analyses in our series was 0.1 mg /.

#### Lactate

The method of BARKER & SUMMERS (1941) was used. The samples were obtained as follows: 2 ml blood was drawn in a dry syringe and immediately ejected into a glass tube containing 10  $\mu$ l of 5% heparin solution. After mixing, 0.2 ml was pipetted into a glass tube containing an acid cadmium sulfate solution and after vigorous shaking the tube was put in ice. To prevent contamination from the skin the tubes were kept closed with aluminum foil. All determinations were done within 3 hours after sample taking. Racemic lactate was used for preparing standards of 0.50, 1.00, 2.00 and 3.00 mM/L. With each series of determinations the four standards were run and a blank. The maximum difference between duplicate determinations was 0.1 mM/L, the mean difference 0.01 mM/L.

#### Haemoglobin

The alkaline haematocrit method was used (GORTER & DE GRAAFF 1955). The O.D. scale is calibrated against total blood iron (GORTER & DE GRAAFF 1955) by multiplying the iron concentration (in mg / ) with 0.3. Hb concentration (in g / ) is obtained. The maximum difference between duplicate analyses was 0.2 g /.

#### Hb oxygen saturation ( $\text{HbO}_{2, \text{sat}}$ )

We used the two-wavelength spectrophotometric method as described by ZILSTRA & MILLER (1957): the blood haemoglobin layer thickness of 0.013 cm. Following a suggestion of ZILSTRA (1958) O.D. was read at 560 m $\mu$  and 522 m $\mu$ , a Unicam SP 500 spectrophotometer being used. The percentage oxygen saturation is calculated from the

tendency to carbamate formation especially Hb (HENRIQUES, 1928 MARGARIA, 1931 FERGUSON & ROUGHTON 1934<sup>1</sup> 1934<sup>2</sup> ROUGHTON 1935 STADIE & O BRIEN 1937 WYMAN 1948 ROUGHTON 1954) In the case of the plasma proteins this tendency is very much smaller As direct determination of carbamino-compound in plasma has not been possible (ROUGHTON 1962) STADIE & O BRIEN (1937) used an indirect method. But the value at which they arrive for plasma at physiological pH 38°C and normal  $P_{CO_2}$  (i.e. 0.3 to 0.5 mM/L  $CO_2$  bound as carbamate) is extrapolated from experiments at pH 9.0 and 37°C with a solution containing 30% horse serum protein It should be mentioned that Stadie and O'Brien's values for Hb and HbO<sub>2</sub> carbamate at physiological conditions are about 50% higher than those found by Ferguson and Roughton who used a more direct determination In a personal communication Prof ROUGHTON (1962) has given as his opinion that under physiological conditions 0.5 mM/L must be considered as a maximal value for plasma carbamate. The value is probably smaller since GRISTINA *et al* (1953) concluded from direct determinations on solutions of serumalbumin that at pH values lower than 8.0 at a temperature of 22°C and at physiological  $P_{CO_2}$  the amount of albumin carbamate is negligible The  $\alpha$  NH<sub>2</sub> groups cannot form carbamate to any extent at physiological pH (ROUGHTON 1954), so that it must be assumed that under these conditions only  $\alpha$  NH<sub>2</sub> groups i.e. terminal amino acids of the protein molecule react with  $CO_2$  Since in normal plasma the number of albumin molecules per unit volume is much higher than that of the molecules of other proteins, it is reasonable to assume that the findings of Gristina *et al* preclude that in normal plasma more than 0.2 mM/L of total  $CO_2$  is bound as carbamate.

We have discussed the possible existence of plasma carbamate in some detail, because when larger amounts of the latter are present a significant error will be made in the interpretation of our experimental results. Carbamate represents a negative charge on the protein molecule, and by mistaking it for  $HCO_3^-$  the negative valency of the plasma proteins is underestimated. Moreover Stadie and O'Brien (1937) found both for Hb and HbO<sub>2</sub> a positive linear correlation between protein concentration and carbamate concentration. A similar relation presumably exists in the case of other proteins, and during *in vivo* ultrafiltration any error caused by mistaking carbamate for  $HCO_3^-$  will therefore increase.

On the strength of the arguments put forward we shall assume that the error due to neglecting the existence of carbamate in plasma will lead to overestimating  $HCO_3^-$  concentration by 0.2 mEq/L plasma at pH 7.40 At higher pH values the error should become larger below pH 7.00 it should be negligible (see effect of pH on carbamino-albumin in the publication by GRISTINA *et al*)

The errors due to neglecting the presence of carbonate and the presence of carbamino-protein tend to cancel each other From the estimates made above we conclude that the combined effect of the two errors on the calculation of  $HCO_3^-$  will be negligible at pH values below 7.00 but that it will result in overestimating  $HCO_3^-$  by 0.1 mEq/L plasma at pH 7.40 and by about 0.2 mEq/L plasma at pH 7.80 The error increases when the protein concentration increases

#### Accidental error

The SE of the mean of two duplicate determinations was found to be 0.14 mM/L for the total  $CO_2$  determination whereas the maximum difference between duplicates was 0.005 for the pH determination. Assuming a value of 30.0 mM/L for total  $CO_2$  it can

be calculated that an error of 0.02 in pH (at pH 7.30) will result in an error of 0.2 / in the calculation of bicarbonate concentration. Since in part of the *in vivo* ultrafiltration experiments about 50 / of the pH values have been calculated by interpolation, with a possible error of  $\pm 0.01$  pH unit, the SE of the mean of a duplicate determination presumably is somewhat larger for  $\text{HCO}_3^-$  than for total  $\text{CO}_2$  but it seems unlikely that it is more than 0.20 mEq/L. At pH values below 7.00 however the spread must have been somewhat larger.

#### Partial carbon dioxide tension ( $P_{\text{CO}_2}$ ) in plasma

As we were only interested in the magnitude of the relative changes observed during *in vivo* ultrafiltration, we thought it sufficient to estimate  $P_{\text{CO}_2}$  from total  $\text{CO}_2$  content and pH by means of the line chart published by VAN SLUYKE & SENDROY (1928). The original graph was used as we learned from MÖLLER (1959) that the reproduction of the graph in PETERS & VAN SLUYKE (1932) is erroneous.

#### Inorganic phosphate

This was determined as described by DE VRIES in GORTER & DE GRAAFF (1955). After deproteinisation, complex-ion formation with molybdic acid in the presence of ferrous ions is measured colorimetrically at 660–720 m $\mu$ , and compared with standard solutions (2.5 mg / and 4.0 mg / P). The maximum difference between duplicate analyses in our series was 0.1 mg /.

#### Lactate

The method of BARKER & SUMNERSON (1941) was used. The samples were obtained as follows: 2 ml blood was drawn in a dry syringe and immediately ejected into a glass tube containing 10  $\mu$ l of 5 / heparin solution. After mixing, 0.2 ml was pipetted into a glass tube containing an acid cadmium sulfate solution, and after vigorous shaking the tube was put in ice. To prevent contamination from the skin the tubes were kept closed with aluminium foil. All determinations were done within 3 hours after sample taking. Racemic lactate was used for preparing standards of 0.50, 1.00, 2.00 and 3.00 mM/L. With each series of determinations the four standards were run and a blank. The maximum difference between duplicate determinations was 0.1 mM/L, the mean difference 0.01 mM/L.

#### Haemoglobin

The alkaline haematin method was used (GORTER & DE GRAAFF 1955). The O.D. scale is calibrated against total blood iron (GORTER & DE GRAAFF 1955) by multiplying the iron concentration (in mg %) with 0.3. Hb concentration (in g %) is obtained. The maximum difference between duplicate analyses was 0.2 g %.

#### Hb oxygen saturation (HbO<sub>2</sub> %)

We used the two-wavelength spectrophotometric method as described by ZILSTRA & MULLER (1957), the blood having a layer thickness of 0.013 cm. Following a suggestion of ZILSTRA (1958) O.D. was read at 560 m $\mu$  and 522 m $\mu$ , a Unicam SP 500 spectrophotometer being used. The percentage oxygen saturation is calculated from the



tendency to carbamate formation especially Hb (HENRIQUES 1928 MARGARIA, 1931 FERGUSON & ROUGHTON 1934<sup>1</sup> 1934<sup>2</sup> ROUGHTON 1935 STADIE & O BRIEN 1937 WYMAN 1948 ROUGHTON 1954) In the case of the plasma proteins this tendency is very much smaller As direct determination of carbamino-compound in plasma has not been possible (ROUGHTON 1962) STADIE & O BRIEN (1937) used an indirect method. But the value at which they arrive for plasma at physiological pH 38 C and normal  $P_{CO_2}$  (i.e. 0.3 to 0.5 mM/L  $CO_2$  bound as carbamate) is extrapolated from experiments at pH 9.0 and 0 C with a solution containing 30% horse serum protein It should be mentioned that Stadie and O'Brien's values for Hb and  $HbO_2$  carbamate at physiological conditions are about 50% higher than those found by Ferguson and Roughton who used a more direct determination In a personal communication Prof. ROUGHTON (1962) has given as his opinion that under physiological conditions 0.5 mM/L must be considered as a maximal value for plasma carbamate The value is probably smaller since GIUSTINA *et al.* (1953) concluded from direct determinations on solutions of serumalbumin that at pH values lower than 8.0 at a temperature of 22 C and at physiological  $P_{CO_2}$  the amount of albumin carbamate is negligible The  $\epsilon$   $NH_2$  groups cannot form carbamate to any extent at physiological pH (ROUGHTON 1954), so that it must be assumed that under these conditions only  $\alpha$   $NH_2$  groups i.e. terminal amino acids of the protein molecule react with  $CO_2$  Since in normal plasma the number of albumin molecules per unit volume is much higher than that of the molecules of other proteins, it is reasonable to assume that the findings of Giustina *et al.* preclude that in normal plasma more than 0.2 mM/L of total  $CO_2$  is bound as carbamate

We have discussed the possible existence of plasma carbamate in some detail, because when larger amounts of the latter are present a significant error will be made in the interpretation of our experimental results Carbamate represents a negative charge on the protein molecule, and by mistaking it for  $HCO_3^-$  the negative valency of the plasma proteins is underestimated Moreover Stadie and O'Brien (1937) found both for Hb and  $HbO_2$  a positive linear correlation between protein concentration and carbamate concentration A similar relation presumably exists in the case of other proteins, and during *in vivo* ultrafiltration any error caused by mistaking carbamate for  $HCO_3^-$  will therefore increase.

On the strength of the arguments put forward we shall assume that the error due to neglecting the existence of carbamate in plasma will lead to overestimating  $HCO_3^-$  concentration by 0.2 mEq/L plasma at pH 7.40 At higher pH values the error should become larger below pH 7.00 it should be negligible (see effect of pH on carbamino-albumin in the publication by GIUSTINA *et al.*)

The errors due to neglecting the presence of carbonate and the presence of carbamino-protein tend to cancel each other From the estimates made above we conclude that the combined effect of the two errors on the calculation of  $HCO_3^-$  will be negligible at pH values below 7.00 but that it will result in overestimating  $HCO_3^-$  by 0.1 mEq/L plasma at pH 7.40 and by about 0.2 mEq/L plasma at pH 7.80 The error increases when the protein concentration increases

#### Accidental error

The SE of the mean of two duplicate determinations was found to be 0.14 mM/L for the total  $CO_2$  determination whereas the maximum difference between duplicates was 0.005 for the pH determination Assuming a value of 30.0 mM/L for total  $CO_2$  it can

our hands the latter gives values which are on the average 2 / lower but the correlation is a linear one and we may conclude that our data on Hb saturation are reliable to within 3 % over the range 20 to 100 / HbO<sub>2</sub>.

★

### *Summary of chapter III*

Since we will determine NCE by correlating changes in protein concentration with changes in (cat min Cl + HCO<sub>3</sub>) the following conclusions are relevant

The plasma obtained in our experiments was true plasma but for the presence of a known amount of heparin solution. For this a correction can be made.

A small systematic error was found to exist in the determination of Na, K and HCO<sub>3</sub>. For Na and K the error was negative and of the order of respectively 0.07 / and 2 / in normal plasma. For HCO<sub>3</sub> it was positive and of the order of 0.3 / in normal plasma. For all 3 substances the error increases with increasing protein concentration. In the case of HCO<sub>3</sub> it also is pH dependent.

We could find no evidence for a systematic error in the determination of water content of plasma, and of the concentrations of protein, Ca, Mg and Cl.

The SE of the mean of a duplicate determination as calculated from about 550 duplicate determinations of plasma, was found to be

Na	0.08 mEq/L	Cl	0.08 mEq/L
K	0.008 mEq/L	HCO <sub>3</sub>	0.20 mEq/L
Ca + Mg	0.012 mEq/L		

For (cat min Cl + HCO<sub>3</sub>), which is calculated from the means of the duplicate determinations of the above mentioned ions, the SD should therefore be

$$\sqrt{0.08^2 + 0.008^2 + 0.012^2 + 0.08^2 + 0.20^2} = 0.25 \text{ mEq/L}$$

This accidental error is independent of protein concentration. The same is true for the error in protein determination, for which the SE of the mean of a duplicate analysis was found to be 0.3 g/L plasma.

It is concluded that the systematic and accidental errors of the analytical techniques are sufficiently small to permit the determination of NCE in the manner to be described in the next chapters.

formula

$$\text{HbO}_2 / \rightarrow \left( a \frac{\text{O D}_{560}}{\text{O D}_{522}} - b \right) 100,$$

in which O D indicates optical density at the wavelength indicated and a and b are constants which are calculated from a series of measurements of O D<sub>560</sub>/O D<sub>522</sub> on solutions containing exclusively Hb or HbO<sub>2</sub>. We refer to ZILSTRA & MULLER (1957) for the argumentation

We could corroborate the mean values given by Zijlstra for the constants a (3.081) and b (1.623) (ZIJLSTRA 1958), but in our hands the spread was fairly large. This is due to the steepness of both absorption curves at the 522 mμ isobestic point. A deviation of 0.5 mμ from the true isobestic point leads to a 2% error in calculating HbO<sub>2</sub>%. Fortunately over the range 520 to 524 mμ the error involved has the same direction and magnitude for HbO<sub>2</sub> and for Hb. We have therefore adopted the following procedure. O D was measured at 520, 522, 524 mμ and at 558, 560, 562 mμ both directly and after saturation of the blood sample with O<sub>2</sub> in a tonometer. Then HbO<sub>2</sub>% of the saturated sample was calculated from

$$\left( 3.081 \frac{\text{O D}_{560}}{\text{O D}_{522}} - 1.623 \right) 100.$$

Generally 100 / ± 1 was found, but if a lower or higher value was obtained, that wavelength was accepted as isobestic point, the O D of which gave a value of 100% when inserted in the abovementioned equation. The same wavelength was then used for calculating the Hb saturation of the original samples, the precise O D being obtained by interpolation from the O D measured at the six wavelengths mentioned above. With this indirect manner of checking, the apparent isobestic wavelength was found to vary between 522 and 523 mμ. When proceeding as described above, we found the duplicate determinations equal to within 2%.

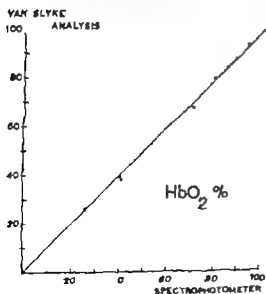


Fig. 18 Determination of percentage HbO<sub>2</sub>. Comparison of manometric method with spectrophotometric method.

Fig 18 shows the correlation between percentage HbO<sub>2</sub> determined in the manner described and that obtained with the manometric method of Van Slyke and Neil. In

our hands the latter gives values which are on the average 2 / lower but the correlation is a linear one and we may conclude that our data on Hb saturation are reliable to within 3 / over the range 20 to 100 / HbO<sub>2</sub>.

★

### *Summary / chapter III*

Since we will determine NCE by correlating changes in protein concentration with changes in (cat min Cl + HCO<sub>3</sub>) the following conclusions are relevant

The plasma obtained in our experiments was true plasma but for the presence of a known amount of heparin solution. For this a correction can be made.

A small systematic error was found to exist in the determination of Na, K and HCO<sub>3</sub>. For Na and K the error was negative and of the order of respectively 0.07 % and 2 / in normal plasma. For HCO<sub>3</sub> it was positive and of the order of 0.3 / in normal plasma. For all 3 substances the error increases with increasing protein concentration. In the case of HCO<sub>3</sub> it also is pH dependent.

We could find no evidence for a systematic error in the determination of water content of plasma, and of the concentrations of proteins, Ca, Mg and Cl

The SE of the mean of a duplicate determination, as calculated from about 550 duplicate determinations of plasma, was found to be

Na	0.08 mEq/L	Cl	0.08 mEq/L
K	0.008 mEq/L	HCO <sub>3</sub>	0.20 mEq/L
Ca + Mg	0.012 mEq/L		

For (cat min Cl + HCO<sub>3</sub>), which is calculated from the means of the duplicate determinations of the above mentioned ions, the SD should therefore be

$$\sqrt{0.08^2 + 0.008^2 + 0.012^2 + 0.08^2 + 0.20^2} = 0.25 \text{ mEq/L}$$

This accidental error is independent of protein concentration. The same is true for the error in protein determination, for which the SE of the mean of a duplicate analysis was found to be 0.3 g/L plasma.

It is concluded that the systematic and accidental errors of the analytical techniques are sufficiently small to permit the determination of NCE in the manner to be described in the next chapters.

*In vivo ultrafiltration*

During his investigation of haemoglobin concentration as an indicator of changes in plasma volume GERBRANDY (1951) studied the effect of upper arm compression at 90 mm Hg. He became impressed by the magnitude of the increase in protein concentration of the blood taken from a cubital vein and conceived the idea of using this phenomenon for the determination of the protein bound fraction of plasma constituents.

Only recently we learned that the principle has already been used by RECKNAGEL (1930) to demonstrate that bilirubin is bound to plasma proteins, and by VAHLQUEST (1941) to demonstrate the same for serum iron. The latter author actually coined the term *in-vivo-Dialysis*. We also regret that it was not known to us until after the preparation of our manuscript that NORDSTRÖM already in 1955 published the results of an investigation on the dependence of the calcium content of the blood, in which he determined protein-bound calcium in precisely the same manner as we did at a later date (NORDSTRÖM 1955). The authors mentioned have made no attempt to study *in vivo* ultrafiltration in more detail.

GERBRANDY *et al* (1957-1960) found that during venous congestion in accordance with expectation substances not associated with protein (urea, glucose) showed no significant change in concentration per Kg H<sub>2</sub>O whereas the substances known to be completely protein-bound (iron, bilirubin, cholesterol, alkaline phosphatase) showed the same percentage increase as total protein. The percentage increase in Ca and Mg concentration was found to be about 40% of that of protein, the percentage increase in Na concentration about 10% of that of protein. For K this value was near 20%, but after venous congestion K concentration – in contrast to the concentration of the other cations – did not return to its precompression level. It was therefore concluded that the increase in K concentration during venous congestion was partly due to a shift of K from cells (erythrocytes?) to plasma as the result of the concomitant changes in O<sub>2</sub> and CO<sub>2</sub> content of the blood (GERBRANDY *et al* 1960).

In a later study it was demonstrated that, by prolonging the period of venous congestion up to 35 minutes and increasing the number of samples taken, the method could be refined and used for the determination of protein-bound Ca and Mg in the individual person, provided the congested arm was kept completely relaxed (VAN LEEUWEN *et al* 1961). Further evidence was presented to substantiate the conclusion that under these conditions factors such as pH, protein composition and lactate formation remain sufficiently constant to warrant the use of the method for the determination of protein bound Ca and Mg. Preliminary results obtained for Na suggested the possibility of using the method for the determination of the NCE of plasma proteins, provided it could be shown that the increase in difference between the sum concentration of the cations and

the sum concentration of Cl and  $\text{HCO}_3^-$  i.e. (cat. min Cl +  $\text{HCO}_3^-$ ) observed during controlled venous congestion, is a function of the total protein concentration solely.

From earlier experiments and from additional experiments to be reported in this study it was concluded that this is so with certain restrictions. Before considering the evidence for this conclusion (which will entail a study of the effect of upper arm compression on the forearm) we will first describe the technical procedure as used during this study and the manner in which NCE and protein-bound Na, Ca and Mg were calculated from the experimental data.

## IN VIVO ULTRAFILTRATION, TECHNIQUE AND CALCULATIONS

### Technique

All experiments were done between 8.30 and 10.30 a.m. The subjects had not eaten since the evening before, but they were allowed one cup of tea ( $\pm 150$  ml), with about 10 g of sugar at about 7.30 a.m. The normal subjects and the out-patients were instructed to avoid physical exercise as much as possible in the hours preceding the investigation.

On arrival the subject was comfortably placed in a semi-recumbent position with one arm completely relaxed and extended on a cushion. After 15 minutes rest, an 18 gauge needle with pointed stylet was introduced in the median or the ulnar cubital vein of the extended arm following 1 minute compression of the upper arm at 60 mm Hg. The pressure was then immediately released and another 5 minutes were allowed to pass before the first of the *precompression samples* was taken. Generally two, sometimes more, samples were drawn at 3 minutes intervals. Thereafter the upper arm was compressed by inflating a carefully adjusted manometer cuff

In most cases the pressure was 100 mm Hg, but as the few instances in which the systolic pressure was lower used the highest pressure at which the radial pulse could still be felt distinctly. In the present study the pressure in the cuff ranged from 80 to 100 mm Hg.

While keeping the pressure constant within 5 mm Hg, the *compression samples* were taken. In the normal persons their number varied from 5 to 8, and the first sample was generally drawn 5 to 7 minutes after the start of upper arm compression, the other samples were taken at intervals of 2 to 3 minutes, after 20 minutes of compression at intervals of 5 minutes or longer. In most patients sampling was started after 15 minutes and only 3 to 5 compression samples were taken, in order to minimise blood loss. Finally after 30 to 40 minutes compression, the pressure was abruptly released and 2 to 3 *postcompression samples* were drawn. In a number of cases the first was taken within 2 minutes after decompression, but generally 6 minutes were allowed to pass. During the whole period of investigation care was taken that the extended arm remained relaxed.

In the case of the normal subjects the total number of samples was 8 to 13 amounting to a loss of 130 to 220 ml blood, but in the case of the patients - where the total number of samples varied from 6 to 10 - the total amount of blood taken varied from 80 (in case of anaemia) to 180 ml.

The handling of the blood samples has already been described in chapter III. The plasma obtained was always analysed for Na, K, Ca, Mg, Cl, total  $\text{CO}_2$  and total protein. Originally blood pH was determined with every sample, later on with every other sample. In the latter case the pH of the remaining samples was determined by inter

polation so that  $\text{HCO}_3$  could be calculated for all samples. Haemoglobin and haematocrit were determined in at least one precompression and one compression sample. In practically all experiments inorganic phosphate was determined in one of the precompression samples in a number of experiments also in compression samples. The same applies to the determination of the protein fractions, which were determined according to Majoor and in most cases also by paper electrophoresis. In a number of experiments the following analyses were done in one or more samples: blood lactate,  $\text{HbO}_2$ , cholesterol, creatinine, urea. Also in nearly all experiments plasma water content was determined for at least one precompression sample and one sample taken towards the end of the period of compression but generally more samples were analysed. A linear relation between total protein concentration and water content of plasma was calculated for each particular experiment and with its aid all analytical values of the experiment were converted to concentrations per Kg/ $\text{H}_2\text{O}$ .

### Calculations

For each experiment regression lines were calculated for the correlation between the concentration of respectively (cat min  $\text{Cl} + \text{HCO}_3$ ) Na, Ca and Ca + Mg and the

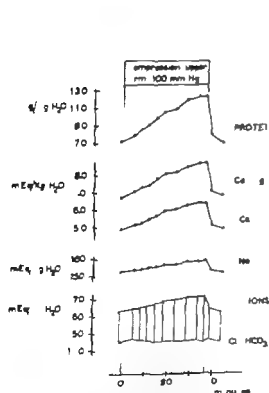


Fig. 19 Changes in concentration as observed in one ultrafiltration experiment.

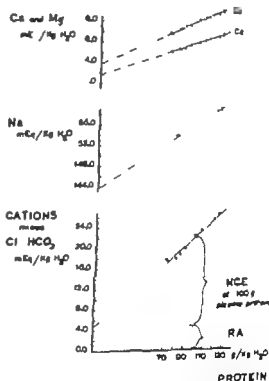


Fig. 20 Graphic representation of manner of calculating NCE and RA (same experiment as Fig. 19).

concentration of protein (method of least squares). From the slope of these regression lines we calculated the NCE per 100 g of total protein respectively the amount of cation bound by 100 g of protein. By extrapolating to protein concentration zero we obtained in the case of (cat min  $\text{Cl} + \text{HCO}_3$ ) the concentration of RA (rest anions) and in the

case of the individual cations the concentration of cation not associated with protein ('free cation').

The procedure is graphically represented in fig. 19 and 20. Fig. 19 shows the changes in the concentration of the ions and of protein as observed in one *in vitro* ultrafiltration experiment, and fig. 20 the correlation between the changes in protein concentration and in ion concentration for the same experiment.

#### A STUDY OF THE SUBJECTIVE AND OBJECTIVE EFFECTS OF PROLONGED COMPRESSION OF THE UPPER ARM

By treating the experimental data in the manner just described, we tacitly assume that during the experiment the concentration of protein is the sole determinant of the concentration of the cations and the sum concentration of  $\text{Cl}$  and  $\text{HCO}_3$ .

For this to be so the plasma samples obtained by means of *in vitro* ultrafiltration must fulfill the following conditions

(1) The correlation between the concentration of a cation e.g. (cat min  $\text{Cl} + \text{HCO}_3$ ) and of plasma protein is linear for protein concentrations ranging from 0 to about 130 g/Kg  $\text{H}_2\text{O}$

(2) The protein composition remains unaltered.

(3) Protein-protein interaction can be neglected.

(4) The concentration of RA does not change.

(5) The effect of the Donnan equilibrium can be neglected

(6) The pH remains constant.

In order to decide whether these conditions indeed exist, we will have to consider in some detail the changes which occur in the forearm during venous compression. We will limit ourselves to the factors which might influence the effectiveness of the *in vitro* ultrafiltration method. For a review of existing knowledge concerning the blood kinetics of the congested limb and the permeability of the capillary wall the reader is referred to LANDIS (1934), who with his collaborators has provided much of the present knowledge on these subjects, and to the interesting article by PAPPENTHIMER (1953). A fairly complete bibliography concerning most aspects of haemoconcentration and haemodilution can be found in Gerbrandy's thesis (1951).

**Blood flow in, appearance of, and sensations in the forearm during venous congestion**  
In the resting and relaxed arm the capillary and venous system is not filled to capacity. On sudden compression of the upper arm at a pressure somewhat below systolic arterial pressure the in-flowing blood will first be accommodated in this latent vascular reservoir. From measurements done by GERBRANDY (1951) it can be seen that as a result of this buffer effect the pressure in the median cubital vein rises only gradually until - after about 5 minutes - it surpasses the compression pressure, and venous blood starts to leave the forearm again. The increase in venous and in capillary pressure has the effect both of increasing ultrafiltration and of decreasing blood flow. The latter phenomenon is the unavoidable corollary of the decrease in arterio-venous pressure gradient. The effect which increasing the venous pressure to 60 mm Hg has on forearm blood flow was studied by several workers with the aid of plethysmography. FISCH *et al* (1950) calculated from their own results and those of HALPERIN *et al*. (1948) a linear relation



which appeared to indicate that at venous pressures higher than 80 mm Hg blood flow would become zero. Actually their own data indicate that the relation is not a linear one. Furthermore the fact that we could compress the upper arm at a pressure of 100–110 mm Hg for periods up to 40 minutes without signs of anaerobic metabolism clearly indicates that even at those high venous pressures some flow must persist. We could not measure the magnitude of the latter with the plethysmographic occlusion method because at 100 mm Hg the venous pressure is too near the systolic arterial pressure. However Dr J Van Noordwijk and Dr T Oei (from the department of Pharmacology University of Amsterdam) were kind enough to determine the blood flow in a relaxed forearm before, during and after compression of the upper arm at 60 mm Hg. One experiment was done and the results are shown in fig. 21

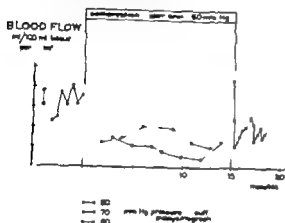


Fig. 21 – Blood flow through forearm before, during and after compression of upper arm (one experiment) See text for explanation.

The subject's systolic arterial pressure was 100 mm Hg. Before compression the collecting pressure in the plethysmograph cuff was 80 mm Hg, after compression it was 70 mm Hg, but during compression two collecting pressures were used alternately in the plethysmograph cuff, to wit 60 and 80 mm Hg. From fig. 21 it will be evident that the intermittent application of the collecting pressure affects the results of the blood flow measurement since a higher blood flow is obtained with the lower collecting pressure.

Although the absolute values obtained for the blood flow are therefore of doubtful significance fig. 21 may serve to indicate the trend, *i.e.* a decrease in blood flow during upper arm compression which levels off after about 5 minutes, with immediately after decompression a sudden and short lasting overshoot this is followed by a longer period in which the blood flow is definitely lower than in the precompression period. The circulatory changes have a rather pronounced effect on the appearance of the congested limb. A detailed description has already been given by AUSPITZ (1874) and later by BIER (1906) and DAUTREBANDE *et al* (1923). Our own observations can be described as follows

In the first minutes of compression a swelling of the superficial veins is seen, followed by an increasingly intense cyanosis of the skin. The latter acquires a mottled appearance with peculiar red patches showing against a bluish background. After about 10 minutes the cyanosis does not further increase, and gradually some edema can be detected. Subjectively the sensation of 'swelling' is most disagreeable between 3 and 10 minutes after the start of compression, when it is often accompanied by itching. Gradually a dulling of the senses occurs, until the arm feels like a *corpus alienum* occasionally a remnant of the itching sensations reminds the owner that he is still in possession. After about 20 minutes a paresis develops and in the few instances in which we have

continued the compression for 30 minutes the forearm and hand muscles were completely paralyzed a similar observation was made by DAUTHENDORF *et al.* (1923) during their experimental study of circulatory stasis. Immediately after decompression the swollen veins are seen to collapse and the skin regains a healthy rosy colour within 2 minutes. But thereafter slight blanching, especially of the fingers, becomes perceptible which may persist for 10 to 15 minutes. Subjectively the first three minutes after decompression are the most unpleasant of the whole experiment. First, hot, tingling sensation creeps through hand and forearm, but this is followed by peculiar cramped feeling in the muscles of the forearm and it needs some force of will to keep the arm and hand relaxed in this period. This lasts for about 2 minutes and then all abnormal sensations disappear. As regards the paresthesia even in the few experiments in which paralysis was induced by prolonging the period of compression, the hand and forearm could be moved without any difficulty within one minute after decompression. Presumably muscle paresthesia during, and the cramped feeling after compression are the result of pressure on the *n. brachialis* and *n. radialis* in the upper arm.

In the more than 120 experiments in which prolonged upper arm compression was applied, Trousseau's sign was only seen twice: once in a patient with hypoparathyroidism and a free calcium concentration of 2.0 mEq/Kg  $H_2O$  and here it lasted only one minute and vanished during the continuation of the compression. The other subject was a healthy young male with completely normal blood chemistry in whom a similar short-lasting Trousseau phenomenon developed during compression. It was never seen in the hypocalcaemic uraemic patients, some of whom had free calcium concentrations lower than 1.6 mEq/Kg  $H_2O$ .

A rather pronounced petechial rash developed in about 40% of the subjects, both in healthy persons and in patients. But contrary to expectation we never saw it in uraemic patients, and in patients with multiple myeloma only to a slight degree. In about 5% of all cases it was very marked, and these were all healthy men. Moreover the tendency to develop a petechial rash was a personal characteristic, since the same persons could always be relied upon to produce a spectacular rash for the benefit of the medical students. The formation of petechiae may have some bearing on the observations concerning protein leakage from the vascular bed during venous congestion (see later).

Finally it should be mentioned that although in some subjects the experiment was often repeated, always using the same arm and sometimes prolonging compression up to 50 minutes, no signs of venous thrombosis were ever observed. Nevertheless, the conditions of the experiment are such that this would appear to be a potential danger. In a few preliminary experiments DEN OTTOLANDER observed an increase in practically all the clotting factors parallel with protein concentration. But on further investigation it was found that the fibrinolytic activity of the venous blood increased even more during compression (DEN OTTOLANDER 1962). Furthermore, Bler who in the beginning of this century introduced prolonged venous congestion as a method of treating chronic inflammatory conditions, did not mention an increased incidence of thrombosis following this treatment nor indeed, any other untoward reaction (BLER 1906). These observations appear to justify the conclusion that no complications are to be feared.

Considering the sensations described above, the method was remarkably well tolerated especially by the patients. It is, however important to explain why the colour of the arm changes, what the duration of the various sensations will be and to engage the subject in a discussion which has his particular interest. At times he is visibly reassured by learning that the investigator underwent the same operation several times and obviously still is in full possession of both his arm and his health. It must be stressed

which appeared to indicate that at venous pressures higher than 80 mm Hg blood flow would become zero. Actually their own data indicate that the relation is not a linear one. Furthermore, the fact that we could compress the upper arm at a pressure of 100-110 mm Hg for periods up to 40 minutes without signs of anaerobic metabolism clearly indicates that even at those high venous pressures some flow must persist. We could not measure the magnitude of the latter with the plethysmographic occlusion method because at 100 mm Hg the venous pressure is too near the systolic arterial pressure. However Dr L. Van Noordwijk and Dr T. Oei (from the department of Pharmacology University of Amsterdam) were kind enough to determine the blood flow in a relaxed forearm before, during and after compression of the upper arm at 60 mm Hg. One experiment was done and the results are shown in fig. 21

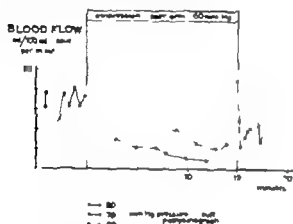


Fig. 21 Blood flow through forearm before, during and after compression of upper arm (one experiment). See text for explanation.

The subject's systolic arterial pressure was 100 mm Hg. Before compression the collecting pressure in the plethysmograph cuff was 80 mm Hg, after compression it was 70 mm Hg, but during compression two collecting pressures were used alternately in the plethysmograph cuff, to wit 60 and 80 mm Hg. From fig. 21 it will be evident that the intermittent application of the collecting pressure affects the results of the blood flow measurement since a higher blood flow is obtained with the lower collecting pressure.

Although the absolute values obtained for the blood flow are therefore of doubtful significance, fig. 21 may serve to indicate the trend, i.e. a decrease in blood flow during upper arm compression which levels off after about 5 minutes, with immediately after decompression a sudden and short lasting overshoot. This is followed by a longer period in which the blood flow is definitely lower than in the precompression period. The circulatory changes have a rather pronounced effect on the appearance of the compressed limb. A detailed description has already been given by AUSPITZ (1874) and later by BEN (1906) and DAUTREBANDE *et al* (1923). Our own observations can be described as follows:

In the first minutes of compression a swelling of the superficial veins is seen, followed by an increasingly intense cyanosis of the skin. The latter acquires a mottled appearance with peculiar red patches showing against a bluish background. After about 10 minutes the cyanosis does not further increase, and gradually some edema can be detected. Subjectively the sensation of swelling is most disagreeable between 3 and 10 minutes after the start of compression, when it is also accompanied by itching. Gradually a dulling of the senses occurs, until the arm feels like a foreign object. Occasionally a remnant of the itching sensations reminds the owner that he is still in possession. After about 20 minutes a paresthesia develops and in the few instances to which we have

continued the compression for 50 minutes the forearm and hand muscles were completely paralysed. A similar observation was made by DAUTREBANDE *et al.* (1923) during their experimental study of circulatory stasis. Immediately after decompression the swollen veins are seen to collapse and the skin regains its healthy rosy colour within 2 minutes. But thereafter a slight blanching, especially of the fingers, becomes perceptible which may persist for 10 to 15 minutes. Subjectively the first three minutes after decompression are the most unpleasant of the whole experiment. First, hot, burning sensation creeps through hand and forearm, but this is followed by a peculiar cramped feeling in the muscles of the forearm and it needs some force of will to keep the arm and hand relaxed in this period. This lasts for about 2 minutes and then all abnormal sensations disappear. As regards the paresthesia even in the few experiments in which a paralysis was induced by prolonging the period of compression, the hand and forearm could be moved without any difficulty within one minute after decompression. Presumably muscle paresthesia during, and the cramped feeling after compression are the result of pressure on the *n. brachialis* and *n. radialis* in the upper arm.

In the more than 120 experiments in which prolonged upper arm compression was applied Trousseau's sign was only seen twice—once in a patient with hypoparathyroidism and a free calcium concentration of 2.0 mEq/Kg  $H_2O$  and here it lasted only one minute and vanished during the continuation of the compression. The other subject was a healthy young male with completely normal blood chemistry in whom a similar short-lasting Trousseau phenomenon developed during compression. It was never seen in the hypocalcaemic uraemic patients, some of whom had free calcium concentrations lower than 1.6 mEq/Kg  $H_2O$ .

A rather pronounced petechial rash developed in about 40% of the subjects, both in healthy persons and in patients. But contrary to expectation we never saw it in uraemic patients, and in patients with multiple myeloma only to a slight degree. In about 5% of all cases it was very marked, and these were all healthy men. Moreover the tendency to develop a petechial rash was a personal characteristic, since the same persons could always be relied upon to produce a spectacular rash for the benefit of the medical students. The formation of petechiae may have some bearing on the observations concerning protein leakage from the vascular bed during venous congestion (see later).

Finally it should be mentioned that although in some subjects the experiment was often repeated, always using the same arm and sometimes prolonging compression up to 50 minutes, no signs of venous thrombosis were ever observed. Nevertheless, the conditions of the experiment are such that this would appear to be a potential danger. In a few preliminary experiments DEN OTTOLANDER observed an increase in practically all the clotting factors parallel with protein concentration. But on further investigation it was found that the fibrinolytic activity of the venous blood increased even more during compression (DEN OTTOLANDER 1962). Furthermore, Bier who in the beginning of this century introduced prolonged venous congestion as a method of treating chronic inflammatory conditions, did not mention an increased incidence of thrombosis following this treatment nor indeed any other untoward reaction (BIER 1906). These observations appear to justify the conclusion that no complications are to be feared.

Considering the sensations described above, the method was remarkably well tolerated especially by the patients. It is, however, important to explain why the colour of the arm changes, what the duration of the various sensations will be and to engage the subject in a discussion which has his particular interest. At times he is visibly reassured by learning that the investigator underwent the same operation several times and obviously still is in full possession of both his arm and his health. It must be stressed

that it is essential to supervise continuously the experimental arm and to ensure its continued relaxation. Only in this way can it be assumed that tissue metabolism remains at a minimum during the whole period of the experiment.

#### The change in protein concentration during compression

In order to obtain a reasonably uniform distribution of experimental points over the range of protein concentrations it was necessary to study the relation between the duration of compression and the increase in protein concentration.

As mentioned before it takes some time before in the relaxed arm the venous pressure exceeds the compression pressure and it is to be expected that the protein concentration

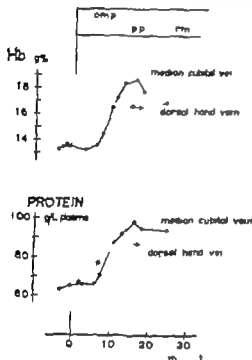


Fig. 22 Changes in Hb- and protein concentration in venous blood sampled from 2 different forearm veins during compression.

will not increase immediately after starting the compression of the upper arm. Moreover the various regions of the vascular bed should differ in this respect. Fig. 22 shows that this is so. The vascular area draining via the dorsal hand vein (mainly skin) has a relatively small latent vascular capacity. As a result the capillary pressure will rise immediately after the start of compression. On the other hand the muscular area from which the median cubital vein receives most of its blood has a large latent vascular capacity with – in the relaxed arm – presumably a rather low tissue pressure (GERBRANDY 1951). This should explain the lag in the increase of Hb and protein concentration observed in median cubital vein blood. But the haemococoncentration ultimately reached for a given compression pressure must be a function of the total filtering area and tissue pressure. Since the relatively avascular and tight dermis cannot compete with muscle tissue in this respect, the blood leaving the latter will in the end reach a higher protein concentration.

In each tissue ultimately equilibrium is established between the increased intracapillary

pressure on the one hand and the increase in colloid osmotic pressure of the plasma and in tissue pressure on the other and no further increase in protein concentration is observed. This is clearly shown in fig. 23 which was constructed from all the *in vivo* ultrafiltration experiments in normal subjects in which samples were taken at regular intervals from 5 to 10 minutes after starting upper arm compression. The percentage increase in protein concentration over the precompression value (concentration in g/Kg  $H_2O$ ) is plotted against time and for every individual experiment the points are connected by lines.

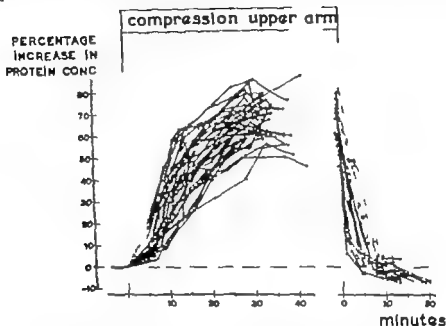


Fig 23 Correlation between duration of compression and increase in protein concentration of venous plasma (sampling one vs cubic)

After 3 to 8 minutes compression the protein concentration starts to increase rapidly but after about 20 minutes a plateau is reached in most cases. After decompression the protein concentration decreases immediately to drop somewhat below precompression values after about 10 minutes. This is presumably the result of reabsorption of accumulated interstitial fluid.

Fig. 23 demonstrates that in healthy subjects the final increase in protein concentration is considerable. However as will be shown later in patients the equilibrium between intra and extravascular pressure is generally reached at a lower level of protein concentration. This phenomenon is apparently not related to the type of the disease. It might be due to the fact that the normal subjects in our study were all relatively young males, whereas the patients included females and were on the average about 50 years old. In these persons the muscle-skin ratio must have been less favourable for reaching high protein concentrations.

In a previous paragraph we have already mentioned the possibility of protein passing the capillary membrane. If the relative increase in haematocrit and in protein concen-

tration during venous compression are compared it is found that at a compression pressure of 60 mm Hg apparently no or practically no protein leaves the plasma, but that at 80 mm Hg a fair amount must pass the capillary membrane (LANDIS *et al* 1932) In Landis experiments compression was continued for 30 minutes, but similar losses of protein were calculated by GERBRANDY (1951) from 2 experiments in which a compression pressure of 90 mm Hg was applied for 8 minutes For 15 of our experiments we calculated the apparent protein loss in the manner described by Landis. The results indicated without exception a considerable loss of protein in some cases, towards the end of compression the loss of water apparently was accompanied by the loss of 50% of the protein which it contained in the plasma. However as a discussion of the possible errors in the necessary calculations would lead too far from the purpose of the present study we will restrict ourselves here to the conclusion that during venous compression under the conditions described a considerable loss of protein may occur presumably via the capillary membrane

This in itself does not prevent the use of controlled venous congestion for the study of ion protein interactions provided that all protein fractions leak to an equal degree. In other words the composition of the plasma proteins must remain unchanged during the whole experiment And this appears to be the case. LANDIS *et al* (1932) already could find no difference in the increase of the albumin and of the globulin fractions (determined according to Howe) GERBRANDY *et al* (1960) using paper electrophoresis, found exactly the same percentage increase for albumin as for total protein after 5 to 10 minutes compression of the upper arm at 90-100 mm Hg. Using pressures of 90 mm Hg for 30 to 40 minutes, we (VAN LEEUWEN *et al* 1961) found an equal percentage increase in protein and in respectively  $^{125}\text{I}$  labelled albumin cholesterol (which might be considered to represent  $\alpha$  and  $\beta$ -globulins) and alkaline phosphatase (which is an  $\alpha_2$ -globulin) For the sake of completeness we have again determined the protein composition with paper electrophoresis during two recent *in vivo* ultrafiltration experiments The changes observed were within the error of the determination (table 23) The conclusion is that whatever protein leaves the capillaries has exactly the same composition as plasma protein In view of the difference in molecular size this is an interesting finding At first sight it appears to indicate that the aqueous channels or pores located in the capillary wall (PAPPENHEIMER 1953) must be larger in diameter than the largest  $\beta$  and  $\alpha$ -globulin molecules. However as was already mentioned in many subjects a petechial rash develops during compression and as these capillary bleedings must also occur in muscle tissue it is conceivable that most of the leakage of plasma proteins occurs through traumatic rents in the capillary wall This is not in contradiction with the observation that relatively more protein leaves the blood than erythrocytes since the latter will only pass through rather large gaps. A further argument for the traumatic origin of the protein loss can be found in the fact that the latter apparently becomes significant only at pressures higher than 60 mm Hg and increases more rapidly than the volume of ultrafiltrate over the range 60 to 80 mm Hg (LANDIS *et al* 1932)

**Changes in  $\text{O}_2$  content  $\text{CO}_2$  content and pH of the venous forearm blood**  
As a result of the decrease in blood flow in the presence of constant tissue metabolism one observes a decrease in oxygen content, a decrease in pH and an increase in  $\text{CO}_2$  content These are induced by respectively a decrease in  $\text{PO}_2$  and an increase in  $\text{PCO}_2$  But

the effect of venous congestion on these parameters is not equally large. Fig. 24 summarizes the results of 17 *in vivo* ultrafiltration experiments (4 patients, 10 normal subjects) in which  $\text{HbO}_2$ , pH and total  $\text{CO}_2$  of plasma were determined in a number of samples before, during and after compression of the upper arm.

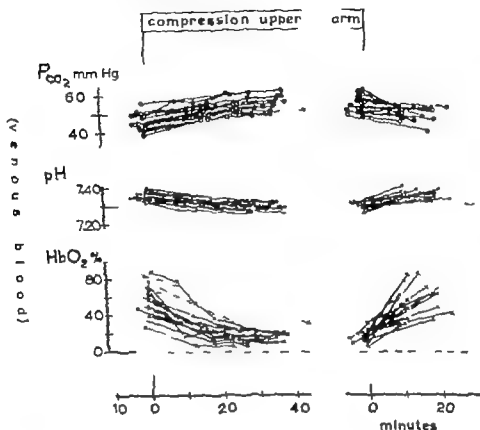


Fig. 24 Correlation between duration of compression and changes in  $P_{\text{CO}_2}$ , pH and percentage  $\text{HbO}_2$  of venous blood (sampling at  $\text{vv}$  cathet).

We have refrained from calculating  $P_{50}$  for the individual experiments because little is known about individual variations in the  $\text{HbO}_2$  dissociation curve.

Fig. 24 demonstrates that, for venous  $P_{\text{CO}_2}$ , pH and  $\text{HbO}_2$  alike, the most pronounced changes occur in the first 15 minutes after starting the compression and in the first 10 minutes after decompression. From 20 minutes after the start of compression onwards the values remain fairly constant. Whereas the maximum change in  $\text{HbO}_2$  was from 60 to 22%, the maximum changes in pH and  $P_{\text{CO}_2}$  were 0.10 pH unit and 14 mm Hg respectively. In fact, the change in pH was so small that we have added Fig. 25 in which the trend in pH is drawn on a much larger scale. It was constructed from the 11 *in vivo*



ultrafiltration experiments in normal subjects, in which the pH was determined with every sample. It demonstrates better than fig. 24 that the main decrease in pH generally occurs in the first 15 minutes of compression.

Table 24 summarises the data represented in fig. 24.

In addition an average value for  $P_{O_2}$  was calculated from the average values for  $HbO_2$  and pH with the aid of the  $HbO_2$  dissociation curve given by DILL (*Handbook of Respiration* 1958).

It would appear that even 10–15 minutes after decompression the values have not yet reached the precompression levels. This is in agreement with the observation that after decompression the blood flow remains below the precompression value.

Although there is an inverse relationship between the total blood flow through the forearm and the arterial venous (A-V) difference in blood gas content, the latter cannot

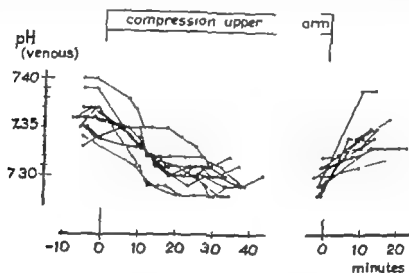


Fig. 25 - Correlation between duration of compression and change in pH of venous blood.

in our experiments be used for calculating the former even if tissue metabolism is kept constant and anaerobic metabolism prevented by completely relaxing the forearm muscles. This is due to the fact that the samples were taken from a cubital vein. During congestion the relative quantities of blood passing through muscle, through skin and through bone will most certainly alter and thereby cause changes in the composition of cubital vein blood independent of changes in total forearm blood flow. It is nevertheless interesting to have some idea of the changes which occur in A-V difference. The more so because we were initially somewhat alarmed by the fact that the change in A-V difference appeared to be definitely larger for  $O_2$  than for  $CO_2$ . In two healthy subjects and in one patient with chronic uraemia but with a haemoglobin concentration of 12 g we calculated the A-V differences for  $O_2$  and for  $CO_2$  before, during and after compression (at 90–110 mm Hg) and from these data the apparent respiratory quotient was obtained. Fig. 26 shows the result.

The values were obtained in the following manner. After the subject had been resting for at least 15 minutes with one arm extended and completely relaxed a needle was introduced in the median cubital vein of the extended arm and a Courmand needle in the brachial artery of the other arm.

A sample of arterial blood was taken at the same time as the first venous sample, thereafter only venous samples were taken. Hb content,  $\text{HbO}_2$  % pH and plasma total  $\text{CO}_2$  concentration were determined in all samples. With the aid of the nomogram, which relates plasma  $\text{CO}_2$  content to blood  $\text{CO}_2$  content (constructed by VAN SLUYK & SUNDLOW (1928)), blood  $\text{CO}_2$  was calculated and expressed in vol %. Blood  $\text{O}_2$  content was calculated from Hb concentration and  $\text{HbO}_2$  % using the factor 1.34 for converting Hb (g%) in oxygen capacity (l of  $\Delta$ ). By assuming the composition of the arterial blood constant for the duration of the experiment A-V differences were calculated for every venous sample, and the apparent respiratory quotient (R.Q.) obtained by dividing the  $\text{CO}_2$  A-V difference by the  $\text{O}_2$  A-V difference.

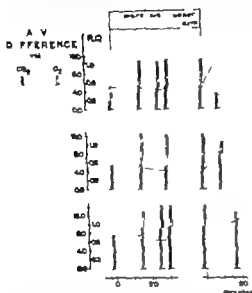


Fig. 26 Correlation between duration of compression and respiratory quotient respectively arterial-venous differences in gas content (venous sampling site vs cubiti).

It can be seen that in every instance the same phenomenon was observed. During compression the  $\text{O}_2$  A-V difference increases more than the  $\text{CO}_2$  A-V difference, but after terminating compression the  $\text{CO}_2$  catches up with the  $\text{O}_2$ . As a result the apparent R.Q. first decreases, but then rises sharply after decompression. In view of the absolute rest of the forearm it is unlikely that changes in tissue metabolism are responsible. In all probability the phenomenon is the result of storage of  $\text{CO}_2$  in interstitial and possibly intra cellular fluid.

After leaving the tissue cells  $\text{CO}_2$  diffuses into the erythrocytes where it is converted into bicarbonate. Each in turn diffuses back again into plasma and tissue fluid. In state of equilibrium the amount of  $\text{CO}_2$  carried away by the venous blood is equal to the amount produced by the cells, and the  $\text{P}_{\text{CO}_2}$  of venous blood and tissues remains constant. But when blood flow decreases, only part of the  $\text{CO}_2$  produced by the cells is carried away. The  $\text{P}_{\text{CO}_2}$  rises and  $\text{CO}_2$  is stored in the forearm tissues. After decompression, when blood flow increases again and end capillary  $\text{P}_{\text{CO}_2}$  drops, the excess tissue  $\text{CO}_2$  mainly present as bicarbonate diffuses back into the plasma and is removed with the venous blood.

The forearm thus acts in precisely the same way as the body as a whole when its steady state in regard to  $\text{CO}_2$  production and transport is disturbed (LILJESTRAND 1916 SHAW 1917 FENN 1928 FARHU & RANV 1955). A similar storage of  $\text{O}_2$  is not possible over the

range of  $P_{O_2}$  observed in our experiments as the myoglobin dissociation curve is hyperbolic in shape and situated far to the left on the  $P_{O_2}$  scale. As a result a decrease in  $P_{O_2}$  from 36 to 15 mm Hg (table 24) would change myoglobin saturation only from about 90% to about 80% (MILLIKAN 1939).

The storage function with regard to  $CO_2$  allows the forearm tissues to accommodate a large amount of  $CO_2$  with a relatively small increase in  $P_{CO_2}$ . This together with the simultaneous conversion of  $HbO_2$  into the weaker acid Hb probably explains the relatively small decrease in venous pH. Table 25 summarises the decrease of pH after 30 minutes compression as observed in a previous study and in the present study. But the change in pH remains small only if the arm is kept relaxed throughout the experiment. One minute rhythmic clenching of the fist during compression of the upper arm at 85 mm Hg was sufficient to decrease the venous pH from 7.28 to 7.14 and to increase blood lactate from 1.2 mM/L to 2.9 mM/L blood (fig. 27).

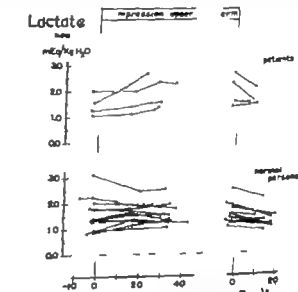
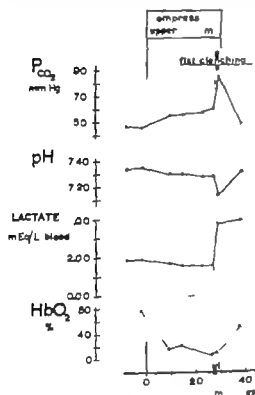


Fig. 28 Effect of compression on lactate concentration of venous plasma (sampling site: venous cannula)

Fig. 27 Effect of muscle contraction on composition of venous blood from forearm (sampling site: median cubital)

### Lactate formation

Complete relaxation is difficult and it might therefore be asked whether during compression no anaerobic metabolism occurs resulting in an increase in lactic acid content of the venous blood. In three healthy persons comparison of lactic acid A-V difference with  $O_2$  A-V difference before, during and after compression for periods up to 35 minutes indicated that no significant increase in lactic acid formation occurred under the conditions of our experiment (Table 3 in VAN LEEUWEN *et al.*, 1961). Furthermore, in 17 experiments (10 normal subjects and 4 patients) venous blood lactate was determined before, during and after compression of the upper arm. The results are shown in fig. 28.

It should be noted that the values are given in mEq per Kg  $H_2O$ . They were calculated from blood values with the aid of the equation  $H_2O$  (g per litre blood) =  $996 - 0.82 (Hb + \text{plasma protein (both in g per litre blood)})$ , where the constants are the same as in the equation which we derived from plasma water determination as in the ultrafiltration experiments with normal subjects (chapter VI).

In the *normal subjects* when the lactate concentration was low to begin with a slight rise was seen during venous compression but when it was high before compression a slight decrease was observed. After decompression a small decrease occurred in all cases. Table 26 gives the average values obtained in the 13 experiments. We have no explanation for the one experiment in which the value remained well above 2.00 mEq/Kg  $H_2O$ . As far as could be ascertained the subject had not unduly exercised himself before the start of the experiment. However as our building contains no elevator and the experiments were done on the second floor we cannot exclude the possibility that the subject scaled the stairs in a hurry in his eagerness to participate in the experiment.

At any rate, from the fact that both mean value and range are larger before compression than after it must be concluded that the normal subjects had not rested sufficiently long before the experiment started. But the changes in concentration are small enough to support the conclusion that lactate formation must have remained negligible in the normal subjects.

In the four *patients* studied the changes were somewhat larger and the one patient who had anaemia (Hb 9 g %) showed a definite increase during compression. In his case HbO dropped to 7 % and anaerobic metabolism appears to have occurred.

As it was found practically impossible to determine lactate in all *in vivo* ultrafiltration experiments because of the rather laborious technique, we have assumed that in the case of the normal subjects and of the patients with a normal Hb concentration the lactate concentration of the venous blood remained constant. In the few patients with anaemia who were studied, an increase may have occurred during compression.

#### Changes in the concentration of inorganic phosphate

No attempt has been made to determine sulphate or organic anions other than lactate. But inorganic phosphate was studied for several reasons. Quantitatively it is the fourth anion after  $Cl$ ,  $HCO_3$  and protein: it is a representative of intracellular anions and it is reported to be bound by plasma proteins. The latter conclusion was reached by GREECE & POWER (1931) on the strength of the concentration ratio observed in their *in vitro* dialysis experiments, and from their results it would appear that from 10 to 20 % of the inorganic phosphate present in plasma behaves as if it was complex-bound by plasma protein. WALKER (1960), using the ultrafiltration technique of Toribara, calculated that in normal plasma about 25 % of inorganic phosphate must be protein-bound. In plasma of 22 hyperphosphataemic uraemic patients (two of whom had a phosphate concentration higher than 5 mM/L) this fraction was on the average 14 %. But these values are probably too high because of an error in technique. In a more recent publication WALKER (1961) reports that on the average 13 % of inorganic phosphate is protein-bound in normal persons. The results of SEEKLYS (1936), who studied bovine serum by means of ultrafiltration, show no evidence of binding and the observations of HOWKINS *et al.* (1952) appear to indicate that in normal plasma not more than 10 % of inorganic phosphate is bound by the plasma protein.

range of  $P_{O_2}$  observed in our experiments, as the myoglobin dissociation curve is hyperbolic in shape and situated far to the left on the  $P_{O_2}$  scale. As a result a decrease in  $P_{O_2}$  from 36 to 15 mm Hg (table 24) would change myoglobin saturation only from about 90% to about 80% (MILLIKAN 1939).

The storage function with regard to  $CO_2$  allows the forearm tissues to accommodate a large amount of  $CO_2$  with a relatively small increase in  $P_{CO_2}$ . This together with the simultaneous conversion of  $HbO_2$  into the weaker acid Hb probably explains the relatively small decrease in venous pH. Table 25 summarises the decrease of pH after 30 minutes compression as observed in a previous study and in the present study. But the change in pH remains small only if the arm is kept relaxed throughout the experiment. One minute rhythmic clenching of the fist during compression of the upper arm at 85 mm Hg was sufficient to decrease the venous pH from 7.28 to 7.14 and to increase blood lactate from 1.2 mM/L to 2.9 mM/L blood (fig. 27).

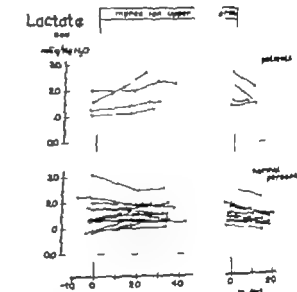
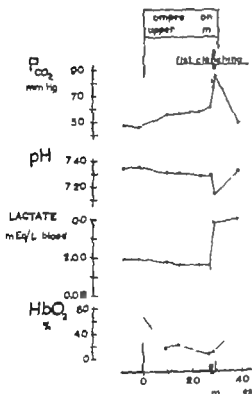


Fig. 28 Effect of compression on lactate concentration of venous plasma (sampling site: vv cubiti)

Fig. 27 Effect of muscle contraction on composition of venous blood from forearm (sampling site: vv cubiti)

### Lactate formation

Complete relaxation is difficult and it might therefore be asked whether during compression no anaerobic metabolism occurs resulting in an increase in lactic acid content of the venous blood. In three healthy persons comparison of lactic acid A-V difference with  $O_2$  A-V difference before, during and after compression for periods up to 35 minutes indicated that no significant increase in lactic acid formation occurred under the conditions of our experiment (Table 3 in VAN LEEUWEN *et al.*, 1961). Furthermore in 17 experiments (10 normal subjects and 4 patients) venous blood lactate was determined before, during and after compression of the upper arm. The results are shown in fig. 28.

The fact that the overall increase in plasma anions is much smaller than the increase in total blood  $\text{CO}_2$  (both expressed per Kg  $\text{H}_2\text{O}$ ) is explained by the effect of the conversion of  $\text{HbO}_2$  into Hb. As a result of the decrease in valencies of the (non-filtrable) haemoglobin molecules the Donnan ratio for the erythrocyte-membrane increases and consequently the sum concentration of Cl and  $\text{HCO}_3$  increases relatively more in the erythrocytes than in the plasma (VAN SLYKE *et al.* 1923; HENDERSON 1931). Moreover the deoxygenation of Hb leads to an increase in the amount of  $\text{CO}_2$  bound as carbamino-haemoglobin (FERGUSON & ROUSSEAU, 1934-35).

As the  $\text{CO}_2$  dissociation curve of blood is practically linear over the range 40 to 60 mm Hg  $\text{Pco}_2$ , we can use DILL's data for estimating the changes to be expected during our experiments. Table 24 shows that in our experiments the  $\text{Pco}_2$  of venous blood increased on the average from 46 mm Hg to 54 mm Hg, *i.e.* 8 mm Hg. On the strength of DILL's values one might therefore expect that during venous compression the pH decreases 0.04, plasma  $\text{HCO}_3$  increases 2.5 mEq, plasma Cl decreases 2.1 mEq and the sum of Cl and  $\text{HCO}_3$  increases 0.4 mEq, all per Kg  $\text{H}_2\text{O}$ . Actually averaging our 34 observations on normal subjects, the changes observed were for pH -0.04, for  $\text{HCO}_3$  +1.3 mEq, for Cl -1.3 mEq, and for Cl+ $\text{HCO}_3$  0.0 mEq per Kg  $\text{H}_2\text{O}$ .

The decrease in pH appears correct but the changes in  $\text{HCO}_3$  and Cl are only half those expected. Even if we acknowledge the fact that DILL *et al.* obtained their values for venous blood from calculations based on *in vitro* experiments, the small increase in plasma  $\text{HCO}_3$  in comparison with the decrease in pH observed in our experiments needs an explanation. Especially so since the decrease in Cl was quantitatively in good agreement with the increase in  $\text{HCO}_3$ .

The discrepancy between pH decrease and  $\text{HCO}_3$  increase might be due to an error in pH measurement as a result of temperature changes in venous blood during compression but experimental evidence (see later) indicates that the average effect of this error on the measured difference in pre- and endcompression pH must have been negligible. The discrepancy is therefore presumably a real one and it suggests the addition to the blood of another acid beside  $\text{H}_2\text{CO}_3$ . It is unlikely that this is an organic acid (in the ordinary sense of the word) since we could detect no significant increase in venous lactate concentration. But an explanation might be provided by the effect of an increase in Hb concentration on the  $\text{CO}_2$  absorption curve of blood (VAN SLYKE *et al.* 1923). Above their isoelectric point haemoglobin and plasma proteins act as acids, with which bicarbonate competes for  $\text{H}^+$ -ions. As a consequence, in  $\text{CO}_2$  absorption curves of blood with normal plasma and cell composition but with increasing haemoglobin concentration, a decrease of bicarbonate concentration per Kg blood should be observed at pH  $\pm 7.30$ . *In vitro* experiments on oxygenated blood of a Mongolian pony confirmed these theoretical considerations (VAN SLYKE *et al.* 1923). In our experiments with normal persons Hb concentration increased on the average 30%, whereas in DILL's calculation Hb concentration was considered to remain constant. It is tempting to estimate with the aid of Van Slyke's data whether the increase in Hb observed in our experiments could have the effect of halving the increase in plasma  $\text{HCO}_3$  to be expected from the observed decrease in pH.

The data obtained on the blood of Mongolian pony in this manner is a hazardous enterprise. Van Slyke *et al.* stress the fact that the absolute values given may differ considerably for other blood samples and that their figures must be considered to indicate merely the relative magnitude of the values expected. However DANTZBACH *et al.* (1923) observed 10% decrease in the  $\text{CO}_2$

In fig. 29 our observations during *in vitro* ultrafiltration are shown. We studied 4 normal persons (the lower values), 1 patient with osteolytic metastases (the median values) and 3 patients with chronic uraemia (the highest values). Only in the case of the highest concentration was a significant change observed and this was a decrease limited to the last sample during compression and the one sample taken after decompression. As a slight decrease in phosphate concentration was to be expected as a result of the change in Donnan equilibrium (to be discussed in a following section) our findings are consistent with the existence of a small amount of protein-bound inorganic phosphate. Its exact magnitude cannot be calculated without knowledge of the magnitude of the change in Donnan ratio but from our results it would appear that it cannot be more than 5% of the inorganic phosphate concentration in normal plasma.

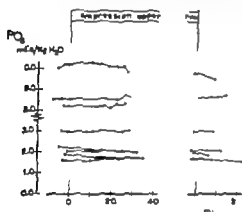


Fig. 29 Effect of compression on concentration of inorganic phosphate in venous plasma (sampling at  $\text{v-v}$  cubit).

#### Changes in the distribution of ions between plasma and erythrocytes - Their effect on plasma $\text{Cl}$ and $\text{HCO}_3$ concentration

During venous congestion the uptake of  $\text{CO}_2$  per unit volume blood increases. After passing into the erythrocytes the  $\text{CO}_2$  is converted into  $\text{H}_2\text{CO}_3$  and this in turn becomes  $\text{HCO}_3$  since the  $\text{H}^+$  ion concentration in the erythrocytes decreases as a result of the conversion of the stronger acid  $\text{HbO}_2$  into the weaker acid  $\text{Hb}$ .

The increase in osmolality resulting from the increase in intracellular  $\text{HCO}_3$  concentration causes the erythrocytes to attract some water. But at the same time  $\text{HCO}_3$  leaves the cells for the plasma and  $\text{Cl}$  moves from the plasma into the erythrocytes, until the concentration ratios for  $\text{HCO}_3$  and  $\text{Cl}$  have regained their normal relation.

These now well known processes have been elucidated by Hamburger by Henderson, Dill, Bock and other associates by Van Slyke and his group and by Roughton and associates. The reader is referred to VAN SLYKE *et al* (1923), HENDERSON (1931), DILL *et al* (1937) <sup>2)</sup> and ROUGHOTON (1954) for qualitative and quantitative data.

For our present problem the difference in composition of arterial and venous blood in resting men found by DILL *et al* (1937) <sup>1)</sup> is of special interest. From their data it can be calculated that in physiological conditions an increase of 6.5 mm Hg in  $\text{Pco}_2$  of the blood is accompanied by a decrease of 0.03 m pH. Simultaneously  $\text{HCO}_3$  content of the serum increases by 1.97 mEq per kg  $\text{H}_2\text{O}$  while  $\text{Cl}$  content decreases by 1.67 mEq per kg  $\text{H}_2\text{O}$ .

equilibrium, the increasing protein concentration of plasma will force the filtrable anions in plasma to decrease in concentration. Whereas the total concentration of the filtrable cations increases, the concentration of cation not associated with protein must decrease parallel with that of the filtrable anions. The magnitude of this effect depends on the Donnan ratio prevailing at the normal protein concentration of plasma and it must affect our calculation of protein-bound cation. This is demonstrated in fig. 30, where the

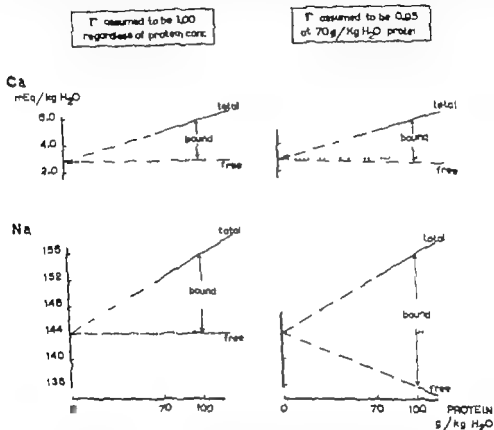


Fig. 30 Effect of the existence of the Donnan-equilibrium on the calculation of the protein-bound and the free fraction of Ca and Na. See text.

Ca and Na regression lines from the experiment depicted in fig. 19 and fig. 20 are reproduced. On the left hand side fig. 30 demonstrates how the amount of cation bound per 100 g of protein is actually calculated i.e. on the assumption that the concentration of free filtrable cation remains constant. In other words, as if the Donnan ratio were 1.000 regardless of the protein concentration in plasma. This picture is clearly erroneous. In reality the concentration of free filtrable cation in plasma decreases with increasing protein concentration as shown in fig. 30 on the right-hand side. As a result the (total) plasma concentration of a cation increases less than the concentration of its protein bound fraction. We can make an estimate of this effect,



combining power of venous forearm blood after 25–40 minutes of stasis, induced by compressing the upper arm at 100–125 mm Hg. In the two experiments shown in their table II the Hb concentration increased by 25 % and in both lactic acid content of the blood remained low and constant.

The conclusions of the authors concerning the *in vivo* changes during their experiments are difficult to follow because pH was calculated from the  $\text{CO}_2$  content of alveolar air and the total  $\text{CO}_2$  of venous blood, using *in vitro* determinations of the  $\text{CO}_2$  absorption curve. But since the latter was determined for both pre- and end-compression samples by equilibrating blood at 37°C against gas of known  $\text{CO}_2$  content, they can be compared. They show that at a pH of about 7.30 the  $\text{CO}_2$  combining power of the blood decreased from 23 to 20 mM/L. with a concomitant increase in Hb from about 80 to 105 %. This change is of the same magnitude as that given by VAN SLYKE *et al* (1923) in their fig. 7 and II suggests that the latter can be used for a quantitative estimate of the effect of Hb concentration on plasma  $\text{HCO}_3$  in our experiments.

This was done in the following manner after recalculating the  $\text{HCO}_3$  concentrations in fig. 7 of VAN SLYKE *et al* (1923) from kg blood to litre blood by using data on the relation between Hb concentration and specific gravity of blood (VAN SLYKE *et al* 1950), one can calculate with the aid of VAN SLYKE and SENDROY's (1928) nomogram (relating plasma  $\text{CO}_2$  content to blood  $\text{CO}_2$  content at varying Hb concentration and oxygen saturation) the effect of increasing Hb on plasma bicarbonate concentration. Thus, one can estimate the change in  $\text{HCO}_3$  concentration per litre plasma, to be expected when pH decreases from 7.35 to 7.30 and  $\text{HbO}_2$  from 60 % to 20 % (very nearly the conditions in our experiments) both for the case that Hb concentration remains constant at 15 g and for the case that Hb concentration changes from 15 g / to 20 g %. In the first instance – i.e. the condition assumed by DILL – plasma  $\text{HCO}_3$  should increase from 23.8 mM/L to 25.2 mM/L, i.e. 1.4 mM/L. In the second case – our conditions – the change should be from 23.8 mM/L to 24.5 mM/L, i.e. only 0.7 mM/L.

It is not suggested that the absolute values thus obtained are accurate, but the result of the calculation indicates that the increase in Hb concentration can explain the apparent discrepancy between the magnitudes of the pH decrease and the  $\text{HCO}_3$  increase observed in our experiments.

It might be asked whether so much reasoning about concentration differences that are after all not larger than about 1.0 mEq is not somewhat ridiculous. The answer must be that it is not. By using the forearm as an apparatus for ultrafiltration we obviously change more factors than those which we aim to use in our final calculation. If no attempt is made to understand all factors and to assess them in at least a roughly quantitative way our final conclusions will remain questionable to a high degree.

Apart from the changes in  $\text{HCO}_3$  and in Cl it is important to consider the change in their sum concentration. As mentioned already in the experiments with normal subjects we found no change during venous compression whereas the sum concentration should have increased about 0.2 mEq as a result of the increased uptake of  $\text{CO}_2$ . The difference is a small one. In fact an astonishingly small one considering the effect which one would expect from the change in Donnan equilibrium between plasma and interstitial fluid. This will be discussed now.

**Changes in the distribution of ions between plasma and interstitial fluid** – The effect of the increase in protein concentration on the Donnan equilibrium

If we assume that the protein concentration of the interstitial fluid is negligible and remains so during venous compression then, according to the dictates of the Donnan

Another assumption was made was that the protein concentration of interstitial fluid remained zero throughout. Earlier investigations appeared to indicate that it was 5 g/L at the highest, but DRINKER and others maintained from their study of the protein concentration of lymph that it could be considerably higher. A review of the literature up to 1934 was given by PETERS (1935), who concluded that Drinker's values were too high. But more recent work favours the concept of a fairly large turnover of proteins between blood and lymph in interstitial fluid and appears to put Drinker in the right (MANNERY 1954). From their experiments on fluid transport across the capillary membrane PAPPENBERGER & SOTO-RIVERA (1948) concluded that the interstitial fluid in skeletal muscle contains about 7 g/L protein. But GERSTANDY (1951) correctly pointed out that in the balance of forces influencing water transport across the capillary membrane the influence of tissue pressure itself was not sufficiently taken into consideration. Consequently the authors underestimated the colloid osmotic effect of interstitial fluid protein and thus protein concentration.

A further point to be realized is that our compression pressures are well above the pressure at which LAMORE *et al.* (1932) found that protein started to leave the blood vessels. Using their method of comparing changes in haematocrit and in total protein, we calculated that considerable amount of plasma protein leaves the blood in the forearm during compression at 100 mm Hg. This suggests that in our experiments the increase in the difference between non-filtrable volumes present on both sides of the capillary membrane is definitely less than will appear from the increase in the intravascular protein concentration alone.

We must conclude that the magnitude of the effect due to changes in Donnan ratio during venous compression remains an open question. In calculating the protein-bound fractions of Na, Ca and Mg probably a certain error will remain. From the fact that we observed no change in the sum concentration of  $\text{Cl} + \text{HCO}_3$  during compression, and assuming 2 mEq of Cl to be complex bound by 70 g normal plasma protein, one would guess that for Na the underestimate will be of the order of 3-4 mEq per 100 g of protein and for Ca about 0.1-0.2 mEq per 100 g protein.

The calculation of NCE is clearly not affected by these changes in Donnan equilibrium since the latter affects the free filtrable cations to the same extent as the free filtrable anions.

#### Changes in cation concentration not caused by the increase in protein concentration

There is no reason to suppose that the free concentration of Ca and Mg changed during our experiments, as their plasma concentrations before and after compression were the same when considered in relation to protein concentration. But it was not always so for Na and practically never so for K.

(1) **Potassium** In earlier experiments (GERSTANDY *et al.*, 1960) it was found that K increased relatively more than Na during venous compression and that its concentration in contrast to that of the other cations did not return to precompression values after compression was released. There is therefore an increase in free K in plasma during venous compression. The cause is not clear. Although apparently in the case of normal erythrocytes no K leaves the cell during increased uptake of  $\text{CO}_2$  from and delivery of  $\text{O}_2$  to the tissues (VANSLYKE *et al.* 1923; TOSTESON *et al.* 1952) it must be doubted whether previous determinations have been accurate enough to detect a systematic increase of the magnitude observed in our experiments. It is also possible that the excess K stems from the tissue cells, in response to the slight acidemia brought about by the transversion of CO into  $\text{H}_2\text{CO}_3$ . The fact that the K concentration remains higher after

If plasma contained no protein the concentration of the cations would be equal on either side of the capillary membrane. As soon as protein is added, filtrable cation and anion move from plasma to interstitial fluid until their concentration ratios across the capillary membrane equal the Donnan ratio for the given protein concentration. The quantity of filtrable ion that must leave the plasma to effect this change in concentration ratio depends on the relative volumes of plasma and interstitial fluid in the forearm. If we assume the latter to be thrice as large as the former (and consider the forearm during venous compression a closed system) the diffusion of a given amount of cation into the interstitial space will decrease plasma concentration thrice as much as it will increase the concentration in interstitial fluid. If we further assume the Donnan ratio to be 0.95 at a protein concentration of 70 g/Kg  $H_2O$  (see chapter II) we find that, while protein increases, free cation and anion decrease until at a protein concentration of 100 g/Kg  $H_2O$  the concentration of free filtrable (univalent) ions in plasma has become 0.947 times their concentration at zero protein. At the same time the concentrations in interstitial fluid should have increased to 1.018 times the initial concentration. Since for the bivalent ions the square of the Donnan ratio must be used, the relative decrease in plasma concentration is more in their case.

For the particular example in fig. 30 we can thus calculate that neglecting the Donnan effect will make us underestimate protein bound Na by 7.5 mEq and protein-bound Ca by 0.30 mEq both per 100 g of protein.

In reality however the Donnan effect must have been smaller under the conditions of our experiments. If fig. 30 (right hand side) were a quantitatively correct representation of the Donnan effect in our experiments, then during compression the sum concentration of Cl and  $HCO_3$  (which was on the average 140 mEq/Kg  $H_2O$ ) should have decreased by about 7.2 mEq for every 100 g increase in protein concentration. As the average increase in protein concentration in the 34 experiments with normal persons was 48 g we must expect the actual decrease to have been 3.5 mEq/Kg  $H_2O$ . Since the extra  $CO_2$  uptake should have increased the sum concentration of Cl and  $HCO_3$  by 0.2 mEq/Kg  $H_2O$  we arrive at the conclusion that according to fig. 30 the overall effect of haemoconcentration on the sum concentration of Cl +  $HCO_3$  should have been a decrease of 3.3 mEq/Kg  $H_2O$ . Instead it was found to be zero.

This discrepancy is only partially explained by the fact that some chloride appears to be bound by albumin at physiological pH. Even if this is also true for conditions *in vivo* not more than 3 mEq of Cl should be complex bound by the amount of albumin present in 100 g normal plasma protein (see chapter I) and the observed 48 g increase in total protein concentration would therefore counteract the influence of the Donnan equilibrium by increasing Cl concentration with 1.5 mEq/Kg  $H_2O$  at the most. This still leaves an expected decrease in Cl +  $HCO_3$  of 1.5 to 2.0 mEq/Kg  $H_2O$ . It would therefore appear that the Donnan effect during venous compression is smaller than that calculated on the assumption of a Donnan ratio 0.95 for normal plasma. In fact if the above reasoning is correct the Donnan ratio must be somewhere between 0.97 and 0.98 when the protein concentration is 70 g/Kg  $H_2O$ . In view of the data discussed in chapter II this seems rather high. But other explanations are also possible.

In the construction of fig. 30 we assumed the interstitial fluid with which the blood in the forearm is in equilibrium to be considerably larger in volume than the plasma. But recent work reviewed by MANDRY (1944) indicates that especially in skeletal muscle the amount of interstitial fluid must be so small that only a thin film will envelop the blood vessels, connective tissue fibres and muscle fibres, and our assumption concerning the relative proportions of plasma and interstitial fluid volume in the forearm must be wrong. This means that for a given change in the Donnan ratio the decrease in the plasma concentration of free filtrable ions will be less, while the increase in their interstitial concentration will be more than was calculated above.

Another assumption we made was that the protein concentration of interstitial fluid remained zero throughout. Earlier investigations appeared to indicate that it was 5 g/L at the highest, but DRINKER and others maintained from their study of the protein concentration of lymph that it could be considerably higher. A review of the literature up to 1934 was given by PETERS (1935), who concluded that Drinker's values were too high. But more recent work favours the concept of fairly large turnover of proteins between blood and lymph via interstitial fluid and appears to put Drinker in the right (MAJERY 1954). From their experiments on fluid transport across the capillary membrane PAPPELMEIER & SOTO-RIVERA (1948) concluded that the interstitial fluid in skeletal muscle contains about 7 g/L protein. But GERRANDY (1951) correctly pointed out that in the balance of forces influencing water transport across the capillary membrane the influence of tissue pressure itself was not sufficiently taken into consideration. Consequently the authors underestimated the colloid osmotic effect of interstitial fluid protein and thus protein concentration.

A further point to be realised is that our compression pressures are well above the pressure at which LAMONT *et al.* (1932) found that protein started to leave the blood vessels. Using their method of comparing changes in haematocrit and in total protein, we calculated that considerable amounts of plasma protein leaves the blood in the forearm during compression at 100 mm Hg. This suggests that in our experiments the increase in the difference between non-filtrable valences present on both sides of the capillary membrane is definitely less than will appear from the increase in the intravascular protein concentration alone.

We must conclude that the magnitude of the effect due to changes in Donnan ratio during venous compression remains an open question. In calculating the protein-bound fractions of Na, Ca and Mg probably a certain error will remain. From the fact that we observed no change in the sum concentration of  $\text{Cl} + \text{HCO}_3$  during compression, and assuming 2 mEq of Cl to be complex-bound by 70 g normal plasma protein, one would guess that for Na the underestimate will be of the order of 3-4 mEq per 100 g of protein and for Ca about 0.1-0.2 mEq per 100 g protein.

The calculation of NCE is clearly not affected by these changes in Donnan equilibrium since the latter affects the 'free' filtrable cations to the same extent as the 'free' filtrable anions.

#### Changes in cation concentration not caused by the increase in protein concentration

There is no reason to suppose that the 'free' concentration of Ca and Mg changed during our experiments, as their plasma concentrations before and after compression were the same when considered in relation to protein concentration. But it was not always so for Na and practically never so for K.

(a) *Potassium* In earlier experiments (GERRANDY *et al.* 1960) it was found that K increased relatively more than Na during venous compression and that its concentration in contrast to that of the other cations - did not return to precompression values after compression was released. There is therefore an increase in 'free' K in plasma during venous compression. The cause is not clear. Although apparently in the case of normal erythrocytes no K leaves the cell during increased uptake of  $\text{CO}_2$  from and delivery of  $\text{O}_2$  to the tissues (VAN SLIKE *et al.* 1923; TOSTESON *et al.* 1952) it must be doubted whether previous K determinations have been accurate enough to detect a systematic increase of the magnitude observed in our experiments. It is also possible that the excess K stems from the tissue cells, in response to the slight acidemia brought about by the transversion of  $\text{CO}_2$  into  $\text{H}_2\text{CO}_3$ . The fact that the K concentration remains higher after

If plasma contained no protein the concentration of the cations would be equal on either side of the capillary membrane. As soon as protein is added, filtrable cation and anion move from plasma to interstitial fluid until their concentration ratios across the capillary membrane equal the Donnan ratio for the given protein concentration. The quantity of filtrable ion that must leave the plasma to effect this change in concentration ratio depends on the relative volumes of plasma and interstitial fluid in the forearm. If we assume the latter to be thrice as large as the former (and consider the forearm during venous compression a closed system) the diffusion of a given amount of cation into the interstitial space will decrease plasma concentration thrice as much as it will increase the concentration in interstitial fluid. If we further assume the Donnan ratio to be 0.95 at a protein concentration of 70 g/Kg  $H_2O$  (see chapter II) we find that, while protein increases, free cation and anion decrease until at a protein concentration of 100 g/Kg  $H_2O$  the concentration of 'free' filtrable (univalent) ions in plasma has become 0.947 times their concentration at zero protein. At the same time the concentrations in interstitial fluid should have increased 1.018 times the initial concentration. Since for the bivalent ions the square of the Donnan ratio must be used, the relative decrease in plasma concentration is more in their case.

For the particular example in fig. 30 we can thus calculate that neglecting the Donnan effect will make us underestimate protein-bound Na by 7.6 mEq and protein bound Ca by 0.30 mEq both per 100 g of protein.

In reality however the Donnan effect must have been smaller under the conditions of our experiments. If fig. 30 (right hand side) were a quantitatively correct representation of the Donnan effect in our experiments, then during compression the sum concentration of Cl and  $HCO_3$  (which was on the average 140 mEq/Kg  $H_2O$ ) should have decreased by about 7.2 mEq for every 100 g increase in protein concentration. As the average increase in protein concentration in the 34 experiments with normal persons was 48 g we must expect the actual decrease to have been 3.5 mEq/Kg  $H_2O$ . Since the extra  $CO_2$  uptake should have increased the sum concentration of Cl and  $HCO_3$  by 0.2 mEq/Kg  $H_2O$  we arrive at the conclusion that according to fig. 30 the overall effect of haemoconcentration on the sum concentration of Cl +  $HCO_3$  should have been a decrease of 3.3 mEq/Kg  $H_2O$ . Instead it was found to be zero.

This discrepancy is only partially explained by the fact that some chloride appears to be bound by albumin at physiological pH. Even if this is also true for conditions *in vivo* not more than 3 mEq of Cl should be complex bound by the amount of albumin present in 100 g normal plasma protein (see chapter I) and the observed 48 g increase in total protein concentration would therefore counteract the influence of the Donnan equilibrium by increasing Cl concentration with 1.5 mEq/Kg  $H_2O$  at the most. This still leaves an expected decrease in Cl +  $HCO_3$  of 1.5 to 2.0 mEq/Kg  $H_2O$ . It would therefore appear that the Donnan effect during venous compression is smaller than that calculated on the assumption of a Donnan ratio 0.95 for normal plasma. In fact if the above reasoning is correct the Donnan ratio must be somewhere between 0.97 and 0.98 when the protein concentration is 70 g/Kg  $H_2O$ . In view of the data discussed in chapter II this seems rather high. But other explanations are also possible.

In the construction of fig. 30 we assumed the interstitial fluid with which the blood in the forearm is in equilibrium to be considerably larger in volume than the plasma. But recent work reviewed by MANERY (1954) indicates that especially in skeletal muscle the amount of interstitial fluid must be so small that only a thin film will envelop the blood vessels, connective tissue fibres and muscle fibres, and our assumption concerning the relative proportions of plasma and interstitial fluid volume in the forearm must be wrong. This means that for a given change in the Donnan ratio the decrease in the plasma concentration of 'free' filtrable ions will be less, while the increase in their interstitial concentration will be more than was calculated above.

MOOK 1931) The reported rates are sufficiently high to guarantee a condition of equilibrium across the capillary wall during the time necessary for taking a sample.

# Effect of venous congestion on the temperature of venous forearm blood - Consequences for the measurement of blood pH

Since inactive muscles produce practically no heat, the temperature of a resting limb depends on the heat apporated by the arterial blood and on its insulation. Even when surrounded by air and when no sweating occurs the latter is not sufficient to prevent a decrease in temperature of the blood during its passage through the limb (HARRIS & MARVIN 1927-1929 ALDENHOVEN & KORTH 1932, HORVATH *et al* 1950 EICHNA *et al* 1951). The blood circulating through skin areas will cool more than the blood flowing through the muscles. As a result the difference in temperature measured between arterial and venous blood must vary according to the area from which the venous blood is drawn. It should be largest in the superficial veins draining the back of the hand. This is indeed so. With copper-constantan thermoelements (soldered into a needle-tip, or introduced into a plastic catheter) the following intravenous temperatures were measured in man (resting and at 20°-24°C ambient temperature)

Dorsal hand vein	34.3	35.8°C	(HARRIS & MARVIN 1927-29)
Forearm vein (cubital)	35.3	36.6°C	(ALDENHOVEN & KORTH 1932)
Vena subclavia	36.0*	36.7°C	(EICHNA <i>et al</i> 1951 if we assume rectal temperature to have been 37.0°C in their subjects).

Furthermore LIPPROSS & SCHEGA (1941) found on one occasion a temperature difference of 1.1°C between art. radialis and vena mediana cubiti.

It follows that the temperature of the blood sampled from the cubital veins during our experiments must have been 1.5 to 2.0 degrees lower than 37.5°C, i.e. the temperature at which all our pH measurements were made. As a consequence our pH values are somewhat too low since measuring pH of whole blood at a temperature higher than the actual temperature of the blood results in a pH decrease of 0.014 to 0.015 unit for every degree Celsius (ROSENTHAL 1948, SIGGAARD ANDERSEN 1963). We found the same relation. But as long as the temperature of the venous blood remains constant during an *in vivo* ultrafiltration experiment, the resulting error in pH measurement will not influence the calculation of NCE. However compression of the upperarm considerably decreases blood flow through the forearm. It is therefore to be expected that the temperature of the venous blood decreases during prolonged compression. Indeed, ALDENHOVEN & KORTH (1932) in fig. 3 of their article show the effect of 3 minutes compression of the upperarm on the temperature in the forearm vein: a decrease from 36.0° to 34.8°C was observed, although the forearm was covered by a cloth. If a similar decrease in venous blood temperature takes place in our experiments, the error made by measuring all samples at 37.5°C will increase during compression, and thus suggest a larger change in pH than actually occurs. For instance if no real change in pH occurs but the temperature drops 2°C, the pH will seemingly decrease by 0.03. As this is about the pH change measured during compression, it was necessary to obtain data on the temperature of the blood as sampled in our experiments.

The temperature was measured intravenously by means of miniature NTC resistance (Philips B 5320 02P/1K) sealed into the tip of closed polyethylene catheter (external diam. 1 mm).

decompression could be explained by the lag in the removal of  $\text{HCO}_3^-$  stored in the tissues during venous compression

This kind of hysteresis phenomenon makes it necessary to consider already here the differences in the overall balance of cations and anions before and after compression. From the 34 observations in normal persons we calculated the mean difference for the sum concentration of cations, for Na, for K, for  $\text{Cl} + \text{HCO}_3^-$ , for protein and for pH. Furthermore the difference in lactate concentration observed in the 13 experiments in normal subjects (table 26) was assumed to be representative for all 34 experiments. The result is shown in table 27

(Cat minus  $\text{Cl} + \text{HCO}_3^- + \text{lactate}$ ) decreased by 0.25 mEq/Kg  $\text{H}_2\text{O}$  pH decreased by 0.01 and there was no change in protein concentration. Assuming the buffer value of plasma protein to be 10 mEq per 100 g protein and per pH unit change (see chapter II), we are left with a difference of 0.15 mEq between pre- and postcompression values which is not accounted for. In view of the cumulative errors involved this may be considered to be non-significant and the result therefore indicates that on the average the NCE before and after venous compression is unchanged. From table 27 it can also be seen that the average change in the cations is solely due to the change in K.

(b) *Sodium* The ingestion of water and of Na and Cl does not occur in a fixed ratio and the adjustment of plasma osmolality is not instantaneous. Therefore occasional changes in the Na (and Cl) concentration of plasma are to be expected which are independent of protein concentration. In our experiments the changes have been minimised by doing all investigations in the morning, allowing at the most 150 ml tea to be taken not later than one hour before the start of the experiment. Only in 8 out of the 34 experiments with normal subjects was a difference of 0.5 mEq/Kg  $\text{H}_2\text{O}$  or more observed between the Na concentration of the pre- and that of the postcompression samples, the largest difference being 1.3 mEq/Kg  $\text{H}_2\text{O}$ . The changes were in both directions and the mean difference between pre- and postcompression samples for the whole group of normal subjects was 0.0 mEq/Kg  $\text{H}_2\text{O}$  (see table 27).

In the 8 cases mentioned the calculation of protein-bound Na will be somewhat in error since one cannot be certain that the change in osmolality was a gradual one. But the calculation of NCE should not be affected since the effect on Na and Cl concentration must have been in the same direction.

In the observations on patients we found the same relative constancy in pre- and postcompression Na values, but owing to the fact that in some patients no postcompression samples were obtained no average change was calculated for this group.

The time necessary for the filtrable ions to establish a new equilibrium between plasma and interstitial fluid, and between plasma and erythrocytes

PAPPENHEIMER (1953) concluded on rather firm grounds that for the small ions the passage across the capillary wall occurs at a rate which is about 50 times higher than the rate at which the ions are apported with the blood. In this respect no difficulties are to be expected during venous compression.

The rate of  $\text{CO}_2$  uptake by and  $\text{H}_2\text{CO}_3$  formation in the erythrocytes appears to be of the order of tenths of seconds, the rate of the Cl shift of the order of seconds (DIRKEN &

pected, the temperature is in the first 10 minutes of compression higher than in the pre and in the postcompression period.

The fact that the change in temperature differs according to the place of sampling may be explained in the following manner. During compression venous blood will start to accumulate in the forearm. Since venous blood is colder than arterial blood but warmer than the surrounding tissues, the temperature in the areas surrounding the main arteries will at first remain unaltered or decrease slightly whereas in the areas away from the main arteries the temperature will at first increase. When the distension of the venous

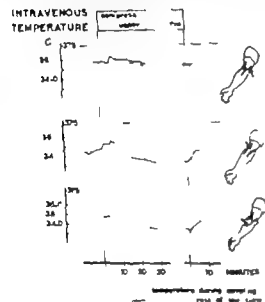


Fig 31 Effect of compression on temperature of blood at different cubital veins. See text.

reservoir has come to an end and compression still continues, the overall loss of heat manifests itself everywhere by a decrease in temperature. The latter will be most pronounced in the areas containing no main artery.

An estimate of the temperature changes that must have occurred during our experiments remains speculative for various reasons. The number of experiments was very small and limited to one person. It is to be expected that the changes will be quantitatively different in different arms. Furthermore, although in most of our experiments the blood was sampled from the ulnar cubital vein, in a number of cases the median cubital vein was chosen for technical reasons. Generally this was not mentioned in the protocol and we are therefore unable to correlate the pH decrease observed in the individual cases with the sampling site. However from the literature and from our own experiments the following conclusions seem justified.

( ) On the average the temperature of the blood at the moment of sampling must have been 1.5 to 2.5°C lower than 37.5°C. Our pH values are therefore on the average 0.03 too low. At the total  $\text{CO}_2$  concentrations observed in our experiments this causes plasma  $\text{HCO}_3^-$  to be underestimated by 0.1 mEq/Kg  $\text{H}_2\text{O}$ .



The NTC resistance (1000  $\Omega$ ) was connected to a recording potentiometer (Variam G10) via a bridge of Wheatstone. At the voltage used (5 V) no heat generation could be detected. The response time of NTC recording system was less than 1" for a temperature change of 2°C the sensitivity was such that 0.1°C corresponded with 1.3 mm deflection over the temperature range 32 to 37°C. The NTC resistance did not respond to mechanical stimuli or changes in the flow of the surrounding fluid (0-20 L/min). The catheter NTC combination could be sterilized by boiling for 20 minutes without changing its temperature-resistance characteristic. The calibration was done immediately after concluding an experiment at several temperatures between 3 and 37°C against the thermometer used in the pH meter circuit (smallest division 0.01°C). The drift was less than 2 mm deflection per hour.

During compression a change in the relative contribution of muscle tissue and of skin to the blood samples is to be expected and since the decrease in temperature must be different in various areas of the forearm, the temperature had to be measured under the conditions prevailing in our experiments.

To that purpose we proceeded in the following manner. A large bore needle fitted with a three way stopcock was used. After introducing the needle in one of the cubital veins with its point in the direction of the forearm, the catheter was introduced through the stopcock opening opposite the needle until the tip containing the NTC resistance projected 0.5 cm into the lumen of the vein. The catheter was then fixed with a Luer Lok adapter so that no leakage could occur. Through the other outlet of the stopcock blood could be sampled. When no samples were drawn the outlet was occluded by a syringe containing a 1/ heparin saline solution. The potential of the NTC chain was continuously recorded with the experimental arm extended and relaxed in the usual manner. The further procedure was exactly as described for the *in vivo* ultrafiltration experiments. After taking 1 or 2 precompression samples the upper arm was compressed for 30 minutes and 4 to 5 samples taken during this period. After releasing the pressure one further sample was taken.

This experiment was repeated twice in the same arm. In the first experiment the needle was introduced into the ulnar cubital vein with the tip of the catheter lying over the brachial artery. In the second experiment it was introduced in the median cubital vein with the catheter tip lying just lateral from the tendon of the biceps. In a third experiment no blood was sampled but the NTC catheter was introduced in the median cubital vein and pushed 5 cm in the direction of the hand. The results of the three experiments are shown in fig. 31. For the first 2 experiments two temperature curves are shown: one indicates the temperature recorded during the time that no samples were taken, the other the temperature measured during sampling.

The first two experiments demonstrate that the temperature recorded during sampling differs from that recorded when no blood flows through the vein in which the NTC resistance is situated.

Moreover the direction of the temperature change is clearly different at the two sites. Near the brachial artery the precompression temperature is relatively high and practically no change is observed during compression, until during the sampling blood is drawn from peripheral areas. Even then the decrease in temperature is not large ( $-0.8^\circ\text{C}$ ). Lateral from the biceps tendon precompression temperature is relatively low and during sampling somewhat lower still. But during compression the temperature first increases until after 10 minutes it starts to decrease. This pattern is most marked during sampling. Although during compression the temperature of the blood samples decreases as ex

$\text{Cl} + \text{HCO}_3$ ) by extrapolating the experimentally determined regression line to zero protein concentration will represent RA *without* NEFA. By subtracting this value from the value found for (cat min  $\text{Cl} + \text{HCO}_3$ ) at 100 g/Kg  $\text{H}_2\text{O}$  protein we neglect the contribution of NEFA to the increase in (cat min  $\text{Cl} + \text{HCO}_3$ ) and overestimate NCE by 0.8 to 0.9 mEq/100 g protein.

However this estimate is based on the assumption that NEFA concentration is determined by protein concentration solely. This is not so. It has become increasingly clear that NEFA is an important source of energy for oxydative processes in muscle tissue (GOODMAN & GORDON 1958) apparently also in the relaxed forearm (ANDRES *et al* 1956, RAMONOWITCH & ZIERLER 1962). A decrease in blood flow might therefore result in an increased A-V difference for NEFA. During *in vivo* ultrafiltration this would counteract the effect of the increase in protein concentration on the NEFA concentration in venous blood. The situation is further complicated by the fact that, whereas muscle tissue takes up NEFA, adipose tissue at the same time releases NEFA. The combined effect of these tissue activities is such that the A-V difference for NEFA can become negative especially when blood is sampled from veins draining skin areas (BALTZAN *et al* 1962). Since it is at present not possible to predict the net effect of the three factors mentioned we will assume that during the *in vivo* ultrafiltration experiments the concentration of NEFA in the venous blood did not change. The available evidence suggests that by this we are more likely to have overestimated NCE (by maximally 1.0 mEq/100 g protein) respectively underestimated RA (by 0.6 mEq/Kg  $\text{H}_2\text{O}$ ) than the reverse.

Furthermore, in patients with anaemia, venous lactate concentration may have increased during compression. Neglect of this change will also have caused us to overestimate NCE and to underestimate RA.

(5) *The influence of the Donnan equilibrium can be neglected.* This is true for the determination of NCE, and nearly true for the determination of protein-bound Ca and Mg. In the case of Na however a rather large error is made which presumably results in underestimating protein-bound Na by about 3-4 mEq per 100 g of protein.

(6) *The pH remains constant.* This assumption is not fulfilled and although the change in pH is small, it is not negligible in its effect on our calculations, as will be demonstrated below.

For every plasma a number of straight lines can be constructed, radiating from one point and representing the relation between protein concentration and NCE for different values of pH. This is shown in fig. 32 for a hypothetical plasma containing 6 mEq/Kg  $\text{H}_2\text{O}$  rest anions. The graph was constructed on the assumption that NCE at pH 7.40 is 24 mEq per 100 g protein, and changes 1.0 mEq/100 g protein/0.1 pH unit (VAN SLIJKE *et al* 1928).

Although the absolute value for NCE found by Van Slyke *et al* has never been corroborated, the change per unit pH seems reasonably well established (chapter II).

The regression lines calculated from our experiments, however, are different in that at the lowest protein concentration (the pre- and postcompression samples) the pH is higher than at the highest protein concentration (the endcompression samples). The experimental points do not therefore lie on a constant pH line, but - because of the roughly

(b) The direction of the change in temperature occurring during compression depends on the site of sampling and apparently it can be as large as 2 C. But on the average in our experiments the difference between the mean temperatures before and after and the temperature at the end of venous compression must have been less than 1 C, and this probably a decrease. This would tend to exaggerate the average decrease in pH during compression by 0.01. It is very unlikely that the error is larger but quite possible that it is smaller and with opposite sign. Although therefore in the individual case one must reckon with an error of about 0.03 in measuring the pH change during compression, the average error for all experiments must have been negligible.

#### FINAL EVALUATION OF THE ASSUMPTIONS MADE WITH REGARD TO THE DATA OBTAINED BY MEANS OF IN VIVO ULTRAFILTRATION

After discussing the changes observed during compression we are now in a position to consider their possible effect on the calculation of protein-bound cation and of the NCE of the plasma proteins.

As stated before the treatment of our experimental data rests on the following suppositions:

(1) *The correlation between the concentration of cation  $c_q$  (cat min  $Cl + HCO_3$ ) and of plasma protein is linear for protein concentrations ranging from 0 to 130 g/Kg  $H_2O$ .* From earlier experiments including equilibrium dialysis we already concluded (VAN LEEUWEN *et al.* 1961) that this is true for Ca, Mg and Na, but not for K. From the fact that during the course of an *in vivo* ultrafiltration experiment the change in the concentration of ( $Cl + HCO_3$ ) is remarkably small, it is already to be expected that (cat min  $Cl + HCO_3$ ) should also show an essentially linear relation with protein concentration. Experimental evidence to be presented in chapter VI and VII indicates that this assumption is sufficiently correct to use the method of extrapolation for calculating NCE in the manner described.

(2) *The protein composition does not change.* This appears to be well established.

(3) *Protein-protein interaction can be neglected.* The fact that the various plasma proteins differ greatly in their IEP leaves open the possibility that the negative charge on some proteins is balanced by positive charge on others. This point will be discussed in some detail in chapter VII where we shall present experimental evidence for the conclusion that in our experiments this effect can be neglected.

(4) *The concentration of RA remains constant throughout the experiment.* The concentration of lactate and inorganic phosphate was indeed remarkably constant in normal subjects and there is no *a priori* reason to expect a change in the concentrations of  $SO_4$ , citrate and the trace anions. For the non-esterified fatty acids (NEFA), however, the situation must be different since these substances are completely bound by plasma protein. The contribution of NEFA to RA should therefore change from nil at a protein concentration zero to about 0.9 mEq/Kg  $H_2O$  at a protein concentration of 100 g/Kg  $H_2O$ . If no other factors influence NEFA concentration the value found for (cat min

Therefore  
RA corrected

$$A - x \text{ (mEq/Kg H}_2\text{O)}$$

NCE corrected

$$(B - A) + (x - z) \text{ (mEq/100 g protein)}$$

The corrected NCE value must be related to the endcompression pH

The smaller the percentage increase in protein concentration has been, the greater the necessary correction will be.

Assuming the NCE of 100 g protein to change 1.0 mEq per 0.1 pH change (see chapter II) we have calculated for all 34 experiments in normal subjects the value of  $x$  and of  $(x - z)$ . It was found that correction of the error due to the change in pH during compression resulted in, on the average 0.9 mEq/Kg  $\text{H}_2\text{O}$  decrease in the value found for RA, and 0.6 mEq increase in NCE per 100 g protein.

For Ca a reasonable estimate of these corrections can be made from the data of TORIYARA *et al* (1957) and of HOPKINS *et al* (1952). Assuming the normal serum or plasma in their experiments to have contained 70 g protein per litre, the work of the authors first mentioned indicates that in the physiological pH range Ca bound per 100 g protein changes 0.12 mEq per 0.1 unit pH whereas from the data of Hopkins *et al* this value is calculated at 0.10 mEq. Accepting therefore for Ca bound per 100 g protein a change of 0.11 mEq per 0.1 unit pH the average value of the extrapolation error due to the pH range is for our group of normal subjects calculated at + 0.09 mEq/Kg  $\text{H}_2\text{O}$  for 'free' Ca, and at -0.06 mEq for Ca bound per 100 g of protein. For Mg the absolute values for the correction must be even smaller and well within the limits of the accuracy of chemical determination.

For Na only a guess can be made, but the absolute value of the error should be somewhat lower than that calculated for NCE.



### Summary of chapter IV

When executed and interpreted in the manner described in this chapter the method of  $\text{in vivo}$  ultrafiltration can be used to determine the NCE of the plasma proteins and protein-bound Ca and Mg with reasonable accuracy. A correction can be made for the error caused by the (small) decrease in pH occurring during compression. The assumption that the concentration of RA does not change during the experiment may lead to overestimating the value of NCE to a small extent (maximally 1 mEq/100 g protein), at least in normal subjects. In patients with anaemia the overestimate of NCE may be larger as a result of an increase in blood lactate during compression. The neglect of the Donnan equilibrium does not affect the determination of NCE and it will cause protein-bound

linear relation between decrease in pH and increase in protein concentration - are spread in a linear fashion over a number of iso-pH lines. By drawing a straight line through the experimental points and extrapolating to protein concentration zero, an error is made which leads to overestimating RA and to underestimating NCE. This is demonstrated by the gross line in fig. 32 which indicates the calculated regression line for a hypothetical experiment with a precompression protein concentration of 70 g/kg H<sub>2</sub>O at pH 7.35 and an endcompression protein concentration of 110 g/Kg H<sub>2</sub>O at pH 7.28

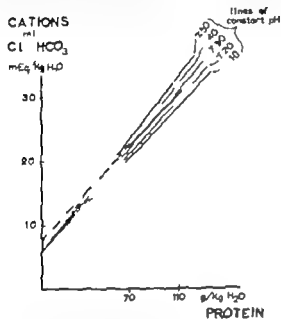


Fig. 32 Systematic error in the determination of NCE and RA resulting from the small decrease in pH during compression. See text

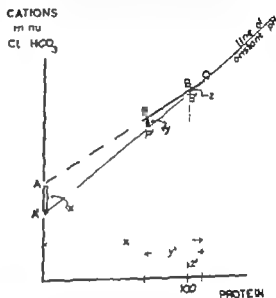


Fig. 33 Scheme explaining correction of the systematic error in NCE and RA caused by the pH change during compression. See text

**Correction for error due to change in pH** It is possible to correct for this error in the manner demonstrated in fig 33 (which is drawn out of proportion for the sake of clearness). Here P and Q represent the extreme points in one experiment. They differ in pH. The straight line drawn through both P and Q is the regression line from which with our mode of calculation RA (indicated as A) and NCE per 100 g protein (indicated as B-A) are obtained. The other line drawn through Q represents the regression line which would have been obtained if for all experimental points the pH had been the same as at Q. The lowest point on the experimental curve would then have been P<sup>1</sup> instead of P. A<sup>1</sup> thus represents the true concentration of RA (B<sup>1</sup> - A<sup>1</sup>) the true NCE for the pH measured at Q i.e. the endcompression pH. It will be evident that  $y (= P - P^1)$  can be calculated from the difference between the pH at P and at Q, the protein concentration at P and the change in NCE per 100 g protein and per unit pH. Furthermore

$$A - A^1 = x = \frac{x^1}{y^1} y \text{ (in mEq/Kg H}_2\text{O)}$$

and

$$B - B^1 = z = \frac{z^1}{y^1} y \text{ (in mEq/Kg H}_2\text{O)}.$$

Therefore  
RA corrected

$$A - x \text{ (mEq/Kg H}_2\text{O)}$$

NCE corrected

$$(B - A) + (x - z) \text{ (mEq/100 g protein)}$$

*The corrected NCE value must be related to the endcompression pH*

The smaller the percentage increase in protein concentration has been the greater the necessary correction will be.

Assuming the NCE of 100 g protein to change 1.0 mEq per 0.1 pH change (see chapter II) we have calculated for all 34 experiments in normal subjects the value of  $x$  and of  $(x - z)$ . It was found that correction of the error due to the change in pH during compression resulted in, on the average, 0.9 mEq/Kg  $\text{H}_2\text{O}$  decrease in the value found for RA, and 0.6 mEq increase in NCE per 100 g protein.

For Ca a reasonable estimate of these corrections can be made from the data of TORIBARA *et al* (1957) and of HOPKINS *et al* (1952). Assuming the normal serum or plasma in their experiments to have contained 70 g protein per litre, the work of the authors first mentioned indicates that in the physiological pH range Ca bound per 100 g protein changes 0.12 mEq per 0.1 unit pH whereas from the data of Hopkins *et al* this value is calculated at 0.10 mEq. Accepting therefore for Ca bound per 100 g protein a change of 0.11 mEq per 0.1 unit pH the average value of the extrapolation error due to the pH range is for our group of normal subjects calculated at + 0.09 mEq/Kg  $\text{H}_2\text{O}$  for Free Ca, and at -0.06 mEq for Ca bound per 100 g of protein. For Mg the absolute values for the correction must be even smaller and well within the limits of the accuracy of chemical determination.

For Na only a guess can be made, but the absolute value of the error should be somewhat lower than that calculated for NCE.



#### *Summary of chapter IV*

When executed and interpreted in the manner described in this chapter the method of *in vivo* ultrafiltration can be used to determine the NCE of the plasma proteins and protein-bound Ca and Mg with reasonable accuracy. A correction can be made for the error caused by the (small) decrease in pH occurring during compression. The assumption that the concentration of RA does not change during the experiment may lead to overestimating the value of NCE to a small extent (maximally 1 mEq/100 g protein), at least in normal subjects. In patients with anaemia the overestimate of NCE may be larger as a result of an increase in blood lactate during compression. The neglect of the Donnan equilibrium does not affect the determination of NCE and it will cause protein-bound

Ca and Mg to be underestimated by about 0.2 and 0.05 mEq/100 g protein only. For protein bound Na, however, the resulting underestimate must be of the order of 3.0 to 4.0 mEq/100 g protein.

The method can not be used for the determination of protein-bound K.

Finally, our experiments confirm the observation of previous authors that the study of venous forearm blood before and during upper arm compression provides useful data concerning the metabolism of the forearm tissues.

*Equilibrium dialysis*

When a protein-containing and a protein-free solution are in equilibrium with each other through a semipermeable membrane, the same molal concentration will be found in both solutions for all filtrable substances not affected by the presence of protein. If on the other hand a substance is bound by protein, its concentration will be higher in the protein-containing solution (MICHAELIS & RONA 1908). Although in the case of electrolytes the Donnan equilibrium is a complicating factor, data obtained by equilibrium dialysis can in principle be used to calculate the amount of protein-bound ion, as well as the fraction that is bound in a complex type manner (chapter I).

In our experiments plasma, serum or a solution containing a purified protein fraction was dialysed at 37.5°C against a synthetic interstitial fluid<sup>1</sup> using cellulose tubing for a semipermeable membrane. The dialysis solution contained Na, K, Ca, Mg, Cl, HCO<sub>3</sub>, CO and inorganic phosphate in physiological concentrations. By using a closed system technique the ratio HCO<sub>3</sub>/CO<sub>2</sub> could be varied and dialysis performed at different pH values. First technique, apparatus and treatment of the experimental data will be described. Thereafter errors in the technique and the observations will be discussed.

## TECHNIQUE AND APPARATUS

## GENERAL OUTLINE

Covered polyethylene containers were filled with dialysis solution having a temperature of 37°-38°C. The containers were mounted on a shaking device inside an incubator (temperature of the latter 37°-38°C) and the pH of the dialysis solution was adjusted by adding appropriate amounts of concentrated HCl. Then the actual dialysis units consisting of a cone-shaped rubber stopper with a bag of cellulose tubing attached to its lower end, were fitted into holes in the covers of the containers so that the bags hung down into the dialysis solution. The bags were subsequently filled with  $\pm 12$  ml of the protein-containing fluid to be studied via a laboratory cannula through a narrow bore channel in the rubber stoppers. One dialysis unit was always filled with distilled water. Immediately after withdrawing the cannula the stoppers were sealed with hardwood plugs and the shaking platform was set in motion. After a period of about 17 hours dialysis was terminated and 1 to 3 samples were taken from the dialysis solution and 1 from the contents of each dialysis unit, using again cannulae and Luer Lok syringes. The transfer to the syringes was done anaerobically with the containers still in the



incubator. During the whole procedure precautions were taken to keep bacterial contamination of the contents of the containers minimal.

All samples were analysed for Na, K, Ca + Mg, Cl, total CO<sub>2</sub>, pH and protein in the manner described in chapter III. Inorganic phosphate and Ca were not routinely determined. One sample of the dialysis solution and at least one sample of the protein-containing fluid were analysed for water content.

Dialysis generally lasted from 16 p.m. to 9 a.m. In some experiments we used one large container with 10 dialysis units, but in most experiments 3 smaller containers were used simultaneously, each accommodating 4 dialysis units and each with a different pH value of its dialysis solution. In our experiments the pH ranged from 6.3 to 8.0.

#### DETAILS CONCERNING APPARATUS AND ITS PREPARATION

##### The polyethylene containers

They are of the ordinary commercial type used in refrigerators. The covers are flexible and fit closely by means of a groove which slips over a rim on the outside of the container. By sealing the connection with several layers of adhesive tape, leakage can be prevented completely. Two sizes of container are used which have the same height (7 cm) but a capacity of 700 ml and of 1300 ml respectively. In the covers round holes (1.5 cm diameter) have been punched out: 5 in the case of the small containers and 11 in the case of the large one.

The containers were prepared for use in the following manner. After carefully fitting the covers on to the containers, the latter were thoroughly rinsed with hot tap water, filled to overflowing with 1% chlorhexidin solution and the holes stoppered with clean rubber stoppers. They were then left to stand for at least 24 hours. Immediately before use the surface of the cover was cleaned with gauze soaked in 1% chlorhexidin solution, the stoppers were removed and the containers emptied by decanting. After at least 2 fillings with boiled distilled water and a final rinsing, the containers were filled with the dialysis solution which had been warmed to a temperature of about 38°C. The containers were then stoppered again, placed in the incubator and further prepared for dialysis as described in the general outline. After completing an experiment the containers were thoroughly cleaned and prepared again in the manner described.

The fact that 1 to 3 ml fluid still remain in the covered containers after the most careful decanting is of no consequence. After 2 fresh fillings with water the chlorhexidin concentration in the 3 ml remaining after the last decanting is 1 p.p.m. and the final concentration of chlorhexidin in the 500 ml dialysis solution must have been about 0.01 p.p.m., i.e. less than  $2 \times 10^{-5}$  mM/L.

##### Dialysis unit

A schematic representation of the small type container with a dialysis unit *in situ* is given in fig. 34. The unit consists of

- 1 cone-shaped rubber stopper with a groove just above its lower end and a narrow channel bored through its length
- 1 piece of seamless cellulose tubing having a length of 8 cm
- 1 diabolo-shaped glass bead
- 2 small size rubber bands
- 1 small cone-shaped piece of polished wood

The dialysis units were assembled immediately before use in the following manner. After boiling stoppers, glass beads and rubber bands several times in distilled water each time refreshing the water, sterile pieces of dialysis tubing, prepared in the manner to be described, were carefully folded with one end around the lower part of a stopper with the other end around a glass bead and secured by small rubber bands. The assembled units were checked for leakage by filling with (sterile) water under a pressure of about 200 mm Hg, and after draining and collapsing the bags by suction they were fitted one

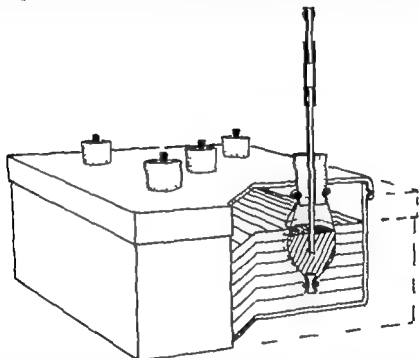


Fig. 14 Polyethylene container with cross-section of dialysis unit and contents (situation during sampling)

by one into the containers inside the incubator. After firmly wedging the rubber stoppers into the holes, the dialysis units were finally filled with the solutions to be dialysed through the stopper channel, which was then plugged with a small cone-shaped piece of polished wood, previously sterilized by boiling. Throughout this procedure the various items were handled with sterile pincers, but the actual assemblage was done manually after the hands had been brushed thoroughly with soap and twice washed with hexachlorophene.

#### Seamless cellulose tubing (Visking)

Throughout the experiments we used size 32/36 (wall thickness 0.001 inch, average pore radius 24 Å). According to the manufacturer the cellulose contains glycerine, water and a small amount of sulphur (approximately 0.1 %) it should be able to withstand steam sterilisation.

The tubing was kept in a polyethylene bag and the day before the start of an experiment pieces of 8 cm length were cut and soaked in distilled water. After some minutes the adhering surfaces could be separated by gently rubbing the tubing between two fingers. The cellulose tubing was then boiled for 20 minutes, rinsed with fresh distilled water and boiled for another 20 minutes. During the night the pieces were left to stand in distilled water and boiled for 15 minutes just before assembling the dialysis units. They were never used twice.

The tubing withstood this treatment without becoming unduly vulnerable and without losing its impermeability to serum proteins, since after 20 hrs dialysis against serum at  $\pm 38^{\circ}\text{C}$  the dialysis solution consistently gave a negative reaction for albumin (as tested by boiling after addition of 1/10 volume of Sørensen's acetic acid acetate buffer; about 0.03 g/L albumin can be detected in this manner).

HUGHES & KLOTZ (1956) using Viking Nojax sausage casing, reported that after heating on a steam-bath for one hour sufficient sulphur was released to colour the water slightly yellow and give it a distinct odour of sulphur. With the more refined type of tubing used in our experiments no such effect was ever seen.

### **Cannulae**

We used 14 gauge 4 laboratory cannulae with a Luer Lok adapter. To prevent air from entering the container when the syringes and cannulae are disconnected the latter have been severed 3 cm below the Luer Lok adapter and the 2 parts connected by a piece of closely fitting rubber tubing which can be compressed by a spring clip (See fig. 34 where the cannula is shown in the position during sampling). Furthermore, to guarantee an equal distribution of the suction force small holes were drilled sideways near the end of the cannula. After use the cannulae were taken apart, thoroughly cleaned after soaking in hot water, reassembled, sterilised by boiling in distilled water and dried by suction.

### **Incubator and shaking device**

The inner dimensions of the incubator are  $50 \times 50 \times 50$  cm. Inside the door a second door of plexiglass has been fitted which can be worked independently. The plexiglass pane is pierced with two holes, each large enough to admit a forearm.

The shaking device consists of a platform which is made to move in a horizontal plane (140 times a minute over a distance of 6 cm) by means of an eccentric transmission. The latter is connected with an electromotor outside the incubator via an axle projecting through its back panel. As a result of this construction the temperature of the solution inside a container placed on the platform can be kept constant to  $0.5^{\circ}\text{C}$ . With every experiment the temperature is checked by means of an (alcohol sterilised) mercury thermometer fitted in one of the containers directly after filling the latter with dialysis solution. In the experiments to be recorded the temperature ranged from  $36.5 - 38.0^{\circ}\text{C}$  at the beginning of the dialysis to  $37.0 - 38.0^{\circ}\text{C}$  at the end. During sampling the temperature inside the containers remained constant to  $0.2^{\circ}\text{C}$ .

# DETAILS CONCERNING THE SOLUTIONS SUBJECTED TO DIALYSIS

## Dialysis solution

During the experiments to be recorded the solutions were at first prepared to contain approximately the following concentrations

Na	144.0	Cl	118.0
K	3.5	HCO <sub>3</sub>	32.0
Mg	1.5	inorganic	
		phosphate	2.0
Ca	3.0	(at pH 7.4)	
<hr/> 152.0 mEq/L		<hr/> 152.0 mEq/L	

Later the K concentration was increased to 5 mEq by adding more KCl. The solution was generally prepared in amounts of 5 litres and to prevent the formation of CaCO<sub>3</sub> the following procedure was adopted. NaCl, KCl, Na HPO and NaHCO<sub>3</sub> were dissolved first the latter in a quantity of 35 mEq/L. Then 3 ml 1 N HCl was added per litre, and the fluid gently mixed in a large stoppered flask. As a result of the formation of H<sub>2</sub>CO<sub>3</sub> the pH decreased only to about 7.40, and CaCl<sub>2</sub> and MgCl<sub>2</sub> could then be added without precipitation of CaCO<sub>3</sub>. A stock of 5 litre solution was sufficient for about 3 dialysis experiments and could be kept in the rubber-stoppered flask at room temperature for a fortnight without precipitation of CaCO<sub>3</sub> and without bacterial growth. Just before starting an experiment the quantity needed was decanted into an Erlenmeyer flask and - without stirring - heated in a waterbath till it had reached a temperature of 40°C. It was then decanted into the containers (500 ± 5 ml in the small ones, 1100 ± 10 ml in the large one) and the containers with the solution immediately placed in the incubator. The pH of the solution was then about 7.80.

Frequent temperature measurements indicated that with this technique the temperature of the solution was between 36° and 38° at the moment of entering the incubator.

Then, according to which pH range was to be studied, 0.1 to 0.9 ml of 10 N HCl was pipetted into the containers and the latter set shaking for about 3 minutes. The CO<sub>2</sub> thus liberated escapes and produces a considerable overpressure. By momentarily opening one of the holes in the cover the pressure inside the containers was allowed to become atmospheric again. Subsequently while placing the dialysis units in position, care was taken that only one hole at a time was open. After some experimenting the intended pH decrease of the solution could be realized to within 0.2 pH unit.

This way of proceeding was found more convenient than the use of solutions with different concentrations of bicarbonate equilibrated against gas containing 5% CO<sub>2</sub> + 95% O<sub>2</sub>.

Since at least one dialysis unit was always filled with distilled water the concentrations in the samples of dialysis solution taken after the dialysis were somewhat lower than those of the stock solution.

### Distilled water

In each container one dialysis unit was always filled with distilled water. This unit served as a control on whether equilibrium had been reached since in that case the concentrations of the filtrable ions will after dialysis be the same for the solution inside the control unit and for the dialysis solution.

### Protein-containing solutions

Apart from the control unit filled with distilled water the dialysis units were filled with protein-containing solutions. In most experiments serum was used, in some plasma. A number of experiments were also done with solutions of purified albumin and  $\gamma$ -globulin. These will be specified when we present our results in chapter VII.

Serum and plasma were obtained in the following manner: within 3 hours before starting the dialysis about 110 ml blood was collected in a sterile small model transfusion flask using the ordinary needle set for donors. When plasma was needed 0.1 ml 5% heparin solution was added beforehand, which made the final concentration of heparin in the plasma between 0.005 to 0.01%. The flask was centrifuged for 60 minutes (2500 rpm at 30°C) and the supernatant pipetted into an Erlenmeyer flask, care being taken that sterile glassware was used throughout. The 50–60 ml serum or plasma obtained were stored at 5°C until they could be transferred to the dialysis unit by means of sterile syringes and cannulae. The maximum capacity of the dialysis bags was about 15 ml, but in the experiments they were filled with  $\pm 12$  ml fluid. In some experiments the protein-containing fluid was diluted to a varying extent with dialysis solution in order to study the effect of changes in protein concentration.

In previous experiments and in the earlier ones of those presented in this study plasma was used. As fibrin frequently formed during the dialysis, only serum was used in the later experiments. With it no late formation of fibrin occurred, at least not visibly. The possibility of differences resulting from the use of either plasma or serum will be discussed when we consider the errors of the method.

The meaning of the terms dialysis solutions, distilled water, serum and plasma. The solutions in the various compartments of a container will change in composition during dialysis. Henceforward the terms dialysis solution, distilled water, serum or plasma will refer to the solutions obtained *after dialysis* unless otherwise stated. The term distilled water is placed between quotation marks since after dialysis the solution inside the control units obviously no longer is distilled water.

## SAMPLING AND TREATMENT OF ANALYTICAL DATA

It was already mentioned that after dialysis the containers were left to stand in the incubator and that sampling was done with the plexiglass door closed. When the large container was used 2 (sometimes 3) samples of dialysis solution were taken, i.e. the first (the fifth) and the last of all samples taken from the container. In the case of the small containers only 1 sample of dialysis solution was obtained and this was the first of the samples taken. The control unit was sampled sixth in sequence in the case of the large container and last in the case of the small containers.

After chemical analysis of the samples and calculating  $\text{HCO}_3^-$  from total  $\text{CO}_2$  and pH

all concentrations were expressed per Kg  $H_2O$  and for each sample (cat min  $Cl + HCO_3$ ) was calculated

For each experiment the correlation between protein concentration and water content was derived from the samples for which water content was determined, i.e. always 1 sample of dialysis solution and at least 1 sample of protein-containing solution (see chapter VI).

Then for Na, K, Ca,  $Ca + Mg$ ,  $Cl$ ,  $HCO_3$  and (cat min  $Cl + HCO_3$ ) the concentration outside the dialysis unit (for which the average concentration in the samples of dialysis solution was taken) was subtracted from the concentration inside each dialysis unit. The values so obtained are indicated by the symbol  $\Delta$  (see chapter I). They were correlated with the protein concentration inside the unit and expressed in mEq per 100 g protein. The values thus found for  $\Delta$  (cat min  $Cl + HCO_3$ ) are considered to represent NCE of the plasma proteins: the values found for  $\Delta Na$ ,  $\Delta K$ ,  $\Delta Ca + Mg$  will be considered as such, since in order to obtain from them the quantity of protein-bound cation they must be corrected for the Donnan equilibrium (chapter I).

Concentration ratios  $([Cat]_e/[Cat]_i)$  and  $[An]_e/[An]_i$  were calculated for each unit,  $[Cat]_e$  and  $[An]_e$  representing the average concentration in the samples of dialysis solution. From the ratios actually found and the protein concentration inside the unit, the concentration ratios to be expected at a protein concentration of 70 g/Kg  $H_2O$  were calculated for each unit (for details see chapter VII). No concentration ratio was calculated for H-ion, for reasons to be explained presently.

## SOURCES OF ERRORS IN TECHNIQUE AND OBSERVATIONS

In the following paragraphs we shall discuss the accidental errors and the various sources for systematic errors.

### ACCIDENTAL ERROR CALCULATED FROM DIFFERENCES IN THE RESULTS OBTAINED FOR COMPARABLE SAMPLES

This we could calculate for two different solutions i.e. the dialysis solution outside and the protein-containing fluid inside the dialysis units.

#### Dialysis solution

Twenty-five experiments are available in which more than one sample of dialysis solution was taken. By subtracting the concentration found in the second sample of dialysis solution from that found in the first sample, the differences in concentration are provided with a sign and the mean value, SD and range of the difference can be calculated. As in later experiments only one sample of dialysis solution was taken, the 25 experiments used in calculating table 28 covered the period between May 1960 and May 1961.

It can be seen that there is no significant difference between the concentrations obtained for consecutive samples of a particular dialysis solution. Taking into consideration the relatively small number of pairs available, the SD is not much different from that calculated for the duplicate determinations of a plasma sample (chapter III). Special attention should be given to the fact that there is no significant difference in pH between the two samples of dialysis solution.

### Distilled water

In each container one dialysis unit was always filled with distilled water. This unit served as a control on whether equilibrium had been reached since in that case the concentrations of the filtrable ions will after dialysis, be the same for the solution made the control unit and for the dialysis solution.

### Protein-containing solutions

Apart from the control unit filled with distilled water the dialysis units were filled with protein-containing solutions. In most experiments serum was used, in some plasma. A number of experiments were also done with solutions of purified albumin and  $\gamma$ -globulin. These will be specified when we present our results in chapter VII.

Serum and plasma were obtained in the following manner: within 3 hours before starting the dialysis about 110 ml blood was collected in a sterile small model transfusion flask using the ordinary needle set for donors. When plasma was needed 0.1 ml 5% heparin solution was added beforehand, which made the final concentration of heparin in the plasma between 0.005 to 0.01 %. The flask was centrifuged for 60 minutes (2500 rpm at 30°C) and the supernatant pipetted into an Erlenmeyer flask, care being taken that sterile glassware was used throughout. The 50–60 ml serum or plasma obtained were stored at 5°C until they could be transferred to the dialysis unit by means of sterile syringes and cannulae. The maximum capacity of the dialysis bags was about 15 ml, but in the experiments they were filled with  $\pm 12$  ml fluid. In some experiments the protein-containing fluid was diluted to a varying extent with dialysis solution in order to study the effect of changes in protein concentration.

In previous experiments and in the earlier ones of those presented in this study plasma was used. As fibrin frequently formed during the dialysis, only serum was used in the later experiments. With it no late formation of fibrin occurred, at least not visibly. The possibility of differences resulting from the use of either plasma or serum will be discussed when we consider the errors of the method.

The meaning of the terms dialysis solutions, distilled water, serum and plasma

The solutions in the various compartments of a container will change in composition during dialysis. Henceforward the terms dialysis solution, distilled water, serum or plasma will refer to the solutions obtained *after dialysis* unless otherwise stated. The term distilled water is placed between quotation marks since after dialysis the solution inside the control units obviously no longer is distilled water.

### SAMPLING AND TREATMENT OF ANALYTICAL DATA

It was already mentioned that after dialysis the containers were left to stand in the incubator and that sampling was done with the plexiglass door closed. When the large container was used 2 (sometimes 3) samples of dialysis solution were taken, i.e. the first, (the fifth) and the last of all samples taken from the container. In the case of the small containers only 1 sample of dialysis solution was obtained and this was the first of the samples taken. The control unit was sampled sixth in sequence in the case of the large container and last in the case of the small containers.

After chemical analysis of the samples and calculating  $\text{HCO}_3^-$  from total  $\text{CO}_2$  and pH

all concentrations were expressed per Kg  $H_2O$  and for each sample (cat min  $Cl + HCO_3$ ) was calculated.

For each experiment the correlation between protein concentration and water content was derived from the samples for which water content was determined, i.e. always 1 sample of dialysis solution and at least 1 sample of protein-containing solution (see chapter VI).

Then for Na, K, Ca, Ca + Mg, Cl,  $HCO_3$  and (cat min  $Cl + HCO_3$ ) the concentration outside the dialysis unit (for which the average concentration in the samples of dialysis solution was taken) was subtracted from the concentration inside each dialysis unit. The values so obtained are indicated by the symbol  $\Delta$  (see chapter I). They were correlated with the protein concentration inside the unit and expressed in mEq per 100 g protein. The values thus found for  $\Delta$  (cat min  $Cl + HCO_3$ ) are considered to represent NCE of the plasma proteins: the values found for  $\Delta Na$ ,  $\Delta K$ ,  $\Delta Ca + Mg$  will be considered as such, since in order to obtain from them the quantity of protein-bound cation they must be corrected for the Donnan equilibrium (chapter I).

Concentration ratios ( $[Cat]_o/[Cat]_i$  and  $[An]_o/[An]_i$ ) were calculated for each unit,  $[Cat]_o$  and  $[An]_o$  representing the average concentration in the samples of dialysis solution. From the ratios actually found and the protein concentration inside the unit, the concentration ratios to be expected at a protein concentration of 70 g/Kg  $H_2O$  were calculated for each unit (for details see chapter VII). No concentration ratio was calculated for H-ion, for reasons to be explained presently.

## SOURCES OF ERRORS IN TECHNIQUE AND OBSERVATIONS

In the following paragraphs we shall discuss the accidental errors and the various sources for systematic errors.

### ACCIDENTAL ERROR CALCULATED FROM DIFFERENCES IN THE RESULTS OBTAINED FOR COMPARABLE SAMPLES

Thus we could calculate for two different solutions i.e. the dialysis solution outside and the protein-containing fluid inside the dialysis units.

#### Dialysis solution

Twenty-five experiments are available in which more than one sample of dialysis solution was taken. By subtracting the concentration found in the second sample of dialysis solution from that found in the first sample the differences in concentration are provided with sign and the mean value, SD and range of the difference can be calculated. As in later experiments only one sample of dialysis solution was taken, the 25 experiments used in calculating table 28 covered the period between May 1960 and May 1961.

It can be seen that there is no significant difference between the concentrations obtained for consecutive samples of a particular dialysis solution. Taking into consideration the relatively small number of pairs available, the SD is not much different from that calculated for the duplicate determinations of a plasma sample (chapter III). Special attention should be given to the fact that there is no significant difference in pH between the two samples of dialysis solution.



Since the mean of the differences did not differ significantly from zero we can consider the SD of the difference given in table 28 to indicate the SD for a single determination of Na etc. in dialysis solution

### Serum or plasma

Since for each dialysis unit the difference in ion concentration between the protein-containing solution inside and the dialysis solution outside is expressed per 100 g protein the values can be compared regardless of the actual protein concentration inside each dialysis unit. By filling two units from the same serum sample, the effect of accidental errors on the value obtained for  $\Delta \text{Na}$   $\Delta \text{K}$  etc. can be estimated. But there is a difference according to whether units from one container are compared or units from different containers. In the first instance only errors in analysis of the inside solution are detected since the outside solution – the dialysis solution – is identical for the units in one container. If on the other hand results from different containers are compared the final error in  $\Delta \text{Na}$   $\Delta \text{K}$  etc. includes also the error inherent in the analysis of dialysis solution.

#### (a) Comparison of results from one container

In practically all experiments at least two dialysis units were filled with the same (undiluted) serum or plasma. A fairly large number of paired results are thus available for studying the effect of accidental errors on the values calculated for  $\Delta \text{Na}$  etc. Since the latter are derived from the differences in concentration inside and outside and multiplied to express them per 100 g of protein the spread must be larger than in the case of the concentrations proper. We have calculated this spread in the following manner. For two paired dialysis units filled with the same serum or plasma the differences in the final  $\Delta$  values are calculated by subtracting the values found for the unit second in sequence of sampling from those found for the unit sampled first. To detect which of the determinations contributes most to the spread in  $\Delta$  (cat min  $\text{Cl} + \text{HCO}_3$ ), we have calculated in the same manner the difference between  $\text{Cl}$  respectively  $\text{HCO}_3$  concentration inside and outside the dialysis unit and expressed it per 100 g of protein. For the differences so obtained the mean, the SD and range were calculated. Results from 54 experiments are available covering the period from May 1960 to December 1961. Table 29 summarises our findings.

It will be seen that again no significant difference is found between the paired samples. The SD of the difference can therefore be considered to indicate the SD for a single determination of  $\Delta \text{Na}$  etc. as well.

The actual SD of  $\Delta$  (cat min  $\text{Cl} + \text{HCO}_3$ ) is smaller than that calculated from the SD of the individual factors ( $\sqrt{0.51^2 + 0.05^2 + 0.10^2 + 0.77^2 + 0.44^2} = 1$ ). This is due to the fact that the spread found for  $\Delta$  cations is not independent from that found for  $\Delta$  anions since the errors in protein determination are the same for both.

A further point of interest is the fact that no significant difference was found between the pH value of the serum-containing-unit sampled first and the one sampled next. Finally it must be stressed that the spread indicated in table 29 is not applicable to the results obtained for dialysis units containing less than 50 g/Kg  $\text{H}_2\text{O}$  of protein. This will be discussed in more detail in one of the following sections.

### (b) Comparison of results from different containers

Since in this case both inside and outside solution are different for the two units compared, both the errors in analysis of serum and in analysis of dialysis solution affect the difference in the values found for  $\Delta Na$  etc.

Table 29 refers to the values obtained by multiplying the experimental results to express  $\Delta Na$  etc. per 100 g protein. In reality however the error in analysis is made when determining the difference between the concentration inside and outside at the actual protein concentration of the solution inside. The latter was on the average 63 g/Kg  $H_2O$  for the experiments in table 29 and the spread to be expected in serum analysis proper is therefore 0.63 SD presented in table 29. From the SD so obtained and the SD for analysis of dialysis solution (table 28) one can make a reasonable estimate of the SD to be expected for a single determination of  $\Delta Na$ ,  $\Delta K$  etc. at a protein concentration of 63 g/Kg  $H_2O$ . The values then found must be multiplied by 100/63 to obtain the SD to be expected for  $\Delta Na$  etc. expressed per 100 g protein (table 30).

Strictly speaking the SD given in table 30 only holds for samples containing 63 g/Kg  $H_2O$  protein. The lower the protein concentration the higher the SD to be expected, because of the multiplication necessary to express the values per 100 g of protein.

But since the values given in table 29 derive from pairs of units with protein concentrations ranging from 52 to 73 g/Kg  $H_2O$  and since the units containing the lowest protein concentration will show the largest spread (and therefore largely determine the SD of the group as a whole), we have accepted the values indicated in tables 29 and 30 as being valid for the experiments in which protein concentration varied between 50 and 75 g/Kg  $H_2O$ . We thereby slightly overestimate the accidental error to be expected at protein concentrations higher than 63 g/Kg  $H_2O$ .

### SYSTEMATIC ERRORS

There are various sources for systematic errors in our experiments. As such we will discuss: The influence of the semipermeable membrane *per se* on ion distribution; the possibility that no equilibrium was reached during dialysis; bacterial contamination; the effect of ageing on ion binding properties of the plasma proteins; differences between plasma and serum; the relation between the actual protein concentration and the values found for  $\Delta Na$  etc. expressed per 100 g protein; and the fact that no anions other than  $Cl$  and  $HCO_3$  were determined.

#### *Influence of the semipermeable membrane per se on ion distribution*

Since we have correlated the difference in the concentration of filtrable ion inside and outside the membrane with the concentration of protein inside, adsorption of ion will have had no effect on our results. Furthermore we found the membrane to be non-selective for all small ions studied (see next section).

#### *Evidence for the existence of a state of equilibrium after 17 hours dialysis*

##### *Filtrable ions other than H-ions*

In preliminary experiments all dialysis units in a container were filled with distilled water and dialysed for 15 hours at 37.5 C against the dialysis solution described before. At the end of that time the solutions inside and outside the bags were always equal, within the error of determination, as regards the concentrations of  $Na$ ,  $K$ ,  $Mg$ ,  $Ca$ ,

of this characteristic of serum, the above mentioned explanation does not answer the question concerning the relatively high pH of serum samples as compared to dialysis solution. We must therefore envisage the possibility that a slight difference in measured pH results from a change in the junction potential when serum is measured instead of saline solution. The above mentioned observations explain why we had to abandon our original intention to use the concentration ratio of H ions as indicator of the Donnan ratio.

All this has no effect on the determination of  $\Delta$  (cat min  $\text{Cl} + \text{HCO}_3$ ) since in the case of  $\text{HCO}_3$  the mean of the differences in concentration does not differ significantly from zero and the distribution of the individual values is symmetrical (fig. 35 below). This

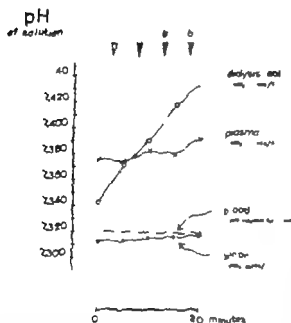


Fig. 36 - Effect of contact with air and of partial vacuum on pH of different solutions.

( ) 2 ml air in syringe containing 5 ml fluid. Expulsion of air after gentle mixing.

(b) 0.5 ml air in syringe containing 4 ml fluid. Creation of partial vacuum by pulling out plunger to 8 ml mark. Expulsion of air.

finding might be interpreted as an argument in favour of the hypothesis that  $\text{CO}_2$  occasionally escapes from the solution during sampling since in that case the resulting decrease in total  $\text{CO}_2$  content and the concomitant increase in pH cancel each other in the calculation of the  $\text{HCO}_3$  concentration.

### Bacterial contamination

All bacteriological examinations were done by Miss J. FASSIA in our Bacteriological Laboratory which is under the direction of Dr. N. LUBSEN.

The conditions during dialysis are ideal for bacteria and the more or less heavy growth of *Ps. aeruginosa* observed in most experiments was not altogether unexpected. But no other bacteria were ever cultured and we were lucky in that the bacteria were only found in the dialysis solution, the contents of the dialysis units remaining remarkable free. This suggests that the aseptic precautions during assembling and filling of the units were adequate and that the dialysis solution was contaminated from either of three sources: the containers, the inside of the incubator and the material from which the dialysis solution was prepared.

The containers seemed highly improbable as a source, since chlorhexidin in a concentration of 1 : 30,000 was found to be bactericidal for this strain of *Ps. aeruginosa*. The inside of the incubator could also be excluded, as cultures from swabs taken from all inner surfaces were consistently negative. Moreover repeatedly washing the inside of the incubator with lysiform did not prevent the growth of *Ps. aeruginosa* during dialysis. Contamination of the stock of dialysis solution appeared also to provide no explanation since in earlier experiments the dialysis solution was autoclaved after its preparation and yet *Ps. aeruginosa* was found at the end of dialysis. We stopped autoclaving the dialysis solutions as being an useless increase in labour and subsequent cultures from the 5 litre stock of dialysis solution always remained negative. Towards the end of the experiment, when all other precautions remained without effect, we again autoclaved the stock solution the first time without result, the two last times no bacterial growth was observed. Since occasionally also no growth occurred while using non-sterilised stock of dialysis solution, we are as yet not certain whether the contamination really occurs during the preparation of the dialysis solution. But after having tried all other measures without avail we are now inclined to think it does. The fact that direct cultures from the stock solution always gave negative results must be explained by the fact that the bacterial density in the dialysis solution remains extremely low until it is dialysed at 38°C and small amounts of nitrogen containing substances (urea) and of glucose diffuse from the serum.

Attempts to prevent bacterial growth by adding oxytetracyclin (30 mg/L) to the dialysis solution were not successful because at 38°C and pH 7.4 oxytetracyclin is broken down within three hours. As *Ps. aeruginosa* was resistant to the other antibiotics available at the time we also tried NaN<sub>3</sub> (0.5 %), but without success. Chlorhexidine in a concentration of 1 : 3000 effectively inhibited the growth, but was found to result in 10-20% error in the determination of Ca and Mg.

For practical purposes it should be mentioned that the macroscopic aspect of the solution was found to be reliable grade in detecting bacterial growth as the latter always caused slight opalescence. If the solution was crystal-clear both the Gram stain of the sediment and the culture were without exception negative.

Although in most of our experiments we were unable to prevent growth of *Ps. aeruginosa* in the dialysis solution, the following points suggest that this has had no effect on our results.

In practically all experiments the contents of the dialysis units remained free from bacteria and since *Ps. aeruginosa* is not known to produce filtrable proteolytic enzymes, it is improbable that the protein inside the dialysis units was altered by the bacterial growth outside.

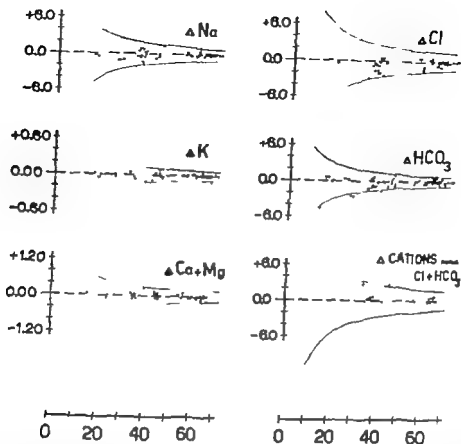
Even in the presence of heavy growth of *Ps. aeruginosa* the potentiometric determination of Cl was not affected. Moreover as already mentioned, equal concentrations were found for all substances inside and outside the control dialysis unit filled with distilled water although the solution inside was always free from bacteria.

#### The effect of ageing on the ion binding properties of the plasma proteins

Normal plasma contains proteolytic enzymes and it is conceivable that these change the number of ionogenic groups on the proteins during the nearly 24 hours dialysis at about 38°C and at physiological pH. We have therefore dialysed serum both when fresh and after it had been stored 1, 3 and/or 7 days at 5°C (3 sera) or at 38°C (1 serum). The results are summarised in fig. 37 and need some explanation.

In view of the effect of pH on the values obtained for  $\Delta Na$  etc. the consecutive dialyses of a given serum should have been performed at exactly the same pH value. But since with our technique the pH realised in a given container sometimes differed as much as 0.2 pH units from the value intended, fig. 37 shows the values obtained for  $\Delta Na$ ,  $\Delta Ca + Mg$  and  $\Delta (cat. min. Cl - HCO_3)$  in relation to the pH values during dialysis.

mEq/100 g protein



ACTUAL PROTEIN CONCENTRATION DIALYSIS UNIT g/Kg  $H_2O$

Fig. 38 Influence of actual protein concentration inside dialysis unit on the  $\Delta$  values expressed in mEq/100 g protein.

Each point represents one dialysis unit the zero lines indicate mean of  $\Delta$  values obtained for undiluted serum the two curved lines accompanying each zero line enclose the area covered by  $\pm 2$  SD for single points.

The relation between the actual protein concentration and the values for  $\Delta Na$ ,  $\Delta K$  etc. expressed per 100 g protein

When we express  $\Delta Na$  etc per 100 g protein we tacitly assume that there is a rectilinear relation between protein concentration and  $\Delta Na$  etc. If this assumption is correct the actual protein concentration inside the dialysis unit will have no influence on the values calculated for  $\Delta Na$  etc per 100 g protein. We have therefore done 10 experiments in which undiluted serum as well as various dilutions of the same serum were simultaneously dialysed in one container.

In each experiment two units contained undiluted serum, the actual concentration of the latter varying from 55 to 71 g/Kg  $H_2O$ . The serum dilutions were made by adding dialysis solutions to the serum. The pH during dialysis varied between 7.10 and 7.50.

The results obtained are presented in the following manner. For each dialysis unit in one container the  $\Delta$  values (in mEq/100 g protein) were subtracted from the mean of the

$\Delta$  values (in mEq/100 g protein) obtained from the two units with undiluted serum in the same container. The differences were plotted against the actual protein concentration in the dialysis unit. The results are for the 10 experiments together shown in fig. 38, where each point represents one dialysis unit.

At first sight the points appear to be equally distributed around the zero line for each of the  $\Delta$  values. In the lower range of the actual protein concentrations however the scatter is considerable. This is explained by the fact that the error due to errors in analysis is magnified by expressing the  $\Delta$  values per 100 g protein. The inevitable increase in spread is the larger the lower the actual protein concentration. In order to know whether the scatter observed is within the range to be expected from errors in analysis we proceeded as follows.

From table 29 we know the SD to be expected for  $\Delta$  Na etc. (expressed per 100 g protein) when the actual protein concentration is 63 g/kg H<sub>2</sub>O and when samples from one container are compared. If we make the assumption that the absolute errors in electrolyte determination are independent from the protein concentration inside the dialysis unit we can calculate the SD to be expected for an actual protein concentration X by multiplying the SD given in table 29 by the factor 63/X. In this manner the curved lines have been constructed which in fig. 38 accompany each zero line. They indicate the range covered by plus or minus 2 SD to be expected for a single point.

Three points (2 for  $\Delta$  Ca + Mg, 1 for  $\Delta$  K) differ more than 3 SD from the zero line and these may therefore lie due to gross error. All other points are within or nearly within 2 SD from the zero line. But whereas for  $\Delta$  Na,  $\Delta$  K,  $\Delta$  Ca + Mg and  $\Delta$  HCO<sub>3</sub> the points are equally distributed around the zero line over the whole protein range, they tend to deviate in the lower range of protein concentration for  $\Delta$  Cl and consequently also for  $\Delta$  (cat min Cl + HCO<sub>3</sub>).

We have therefore also calculated the mean deviation from the zero line for the points within the protein ranges 10-20, 20-30, 30-40 and 40-50 g/Kg H<sub>2</sub>O respectively. For each range the resulting value was compared with 2 SE of the mean to be expected at the average protein concentration for that range. For all other  $\Delta$  values the mean deviation from the zero line was within 2 SE of the mean for each of the protein ranges, but for  $\Delta$  Cl and for  $\Delta$  (cat min Cl + HCO<sub>3</sub>) the same deviation was between 2 SE and 3 SE of the mean in the protein ranges 10-20 and 30-40 g/Kg H<sub>2</sub>O (table 34). The number of points in the lowest range however is only five and in view of the manner in which we had to calculate the SD to be expected at the various protein concentrations we must conclude that the number of experiments is too small to decide whether the observed deviation is statistically significant. If there really is a deviation for  $\Delta$  Cl to lower values and for  $\Delta$  (cat min Cl + HCO<sub>3</sub>) to higher values when the actual protein concentration decreases, the effect should be to underestimate  $\Delta$  Cl and to overestimate  $\Delta$  (cat min Cl + HCO<sub>3</sub>) by expressing them per 100 g protein. However the trend of the points in fig. 38 and the values in table 34 suggest that for experiments with undiluted serum the error would still be less than 0.3 mEq/100 g protein.

Since henceforward we will only consider results obtained with dialysis units containing more than 50 g/Kg H<sub>2</sub>O protein we conclude that in the experiments to be presented later the expression of  $\Delta$  Na,  $\Delta$  K,  $\Delta$  Ca + Mg,  $\Delta$  HCO<sub>3</sub>,  $\Delta$  Cl and  $\Delta$  (cat min Cl + HCO<sub>3</sub>) in mEq/100 g protein did not introduce a systematic error. This is equal to saying that the relation between protein concentration and  $\Delta$  Na etc. is rectilinear for protein concentrations between 50 and 100 g/Kg H<sub>2</sub>O.

**Errors due to the fact that no anions other than Cl and HCO<sub>3</sub> were determined**

The one other anion present in the stock dialysis solution phosphate, was determined only occasionally because of the already considerable amount of analyses. However since both its concentration in the stock solution (2.7-2.9 mg %) and the pH are known, the phosphate concentration in a sample of dialysis solution can be estimated to within 5% at pH 7.40 it should be  $1.6 \pm 0.1$  mEq/kg H<sub>2</sub>O. Furthermore, during dialysis of serum or plasma the filtrable rest anions other than phosphate will be distributed over the total volume of fluid in the container and since maximally 60 ml of serum was dialysed

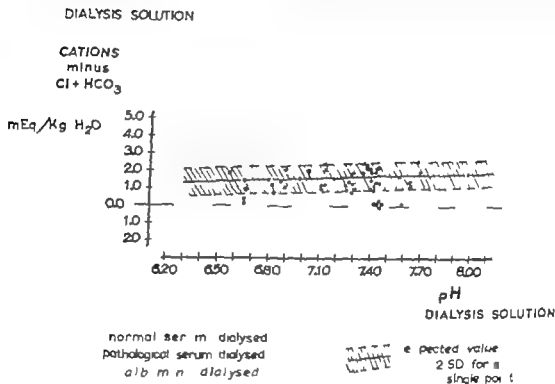


Fig. 39 - Values observed compared with values expected for (cat min Cl + HCO<sub>3</sub>) in dialysis solution. See text.

against 1100 ml dialysis solution the final concentration of these anions is at the most 0.05 times the original serum concentration. If we assume the latter to be 4.0 mEq/Kg H<sub>2</sub>O (chapter II) the final concentration of filtrable anions other than Cl and HCO<sub>3</sub> can be estimated at 1.8 mEq/Kg H<sub>2</sub>O for pH 7.40. We must therefore expect to find this value for (cat min Cl + HCO<sub>3</sub>) in both dialysis solution and distilled water when normal serum is dialysed. Its magnitude is slightly different at other pH values because of the effect of the latter on the dissociation of inorganic phosphate.

In fig. 39 the value expected for (cat min Cl + HCO<sub>3</sub>) in the dialysis solution is compared with the value actually found in the 69 experiments in which plasma, serum or purified protein solutions were dialysed. The continuous line indicates the expected value, each point the actual value found for the dialysis solution. The SD for a single point should be 0.40 mEq/kg H<sub>2</sub>O (see table 28), and the hatched area between the broken lines in fig. 39 encloses all values which differ not more than twice the SD from the value expected.

It is evident that only half the number of experimental points fall within the probable range. Some are higher but most are lower than the expected value and some even carry a negative sign! That these 'aberrations' are greater than the ordinary spread can explain, is corroborated by the fact that in the case of the three extreme values  $+4.6$ ,  $-1.1$  and  $-2.0$ , the values found in the paired samples of 'distilled water' were  $+6.0$ ,  $-1.1$  and  $-2.2$  respectively. Together with the results summarised in table 31 this demonstrates that the deviation from the expected value is found both inside and outside the dialysis units, and to an equal extent. The cause(s) of this phenomenon is (are) not clear.

In view of the data presented in chapter III it is improbable that the discrepancies observed are the result of differences between the standard solutions used for the determination of the cations and of  $\text{Cl}$  and  $\text{HCO}_3^-$ . Moreover, quite often one of the three containers used during one dialysis experiment would yield a value of for instance  $+2.0$  for (cat min  $\text{Cl} + \text{HCO}_3^-$ ), whereas the values found in the other two containers would be zero or even negative. Initially we thought that the differences observed were explained by summation of accidental errors in analysis, but when it became clear that the reproducibility was considerably better than expected, we determined inorganic phosphate in a number of experiments to exclude the possibility of errors from that quarter. Without exception the concentration of inorganic phosphate was found to be within the range  $2.6$ – $2.8$  mg %, although in the same samples the value for (cat min  $\text{Cl} + \text{HCO}_3^-$ ) ranged from  $0.0$  to  $3.5$  mEq/Kg  $\text{H}_2\text{O}$ . Three of the higher values were found in an experiment with serum from a patient with multiple myeloma, but the rest anionic provided no explanation as their concentration in the original plasma was found to be not more than  $8$  mEq/Kg  $\text{H}_2\text{O}$  (see ultrafiltration method).

If we reject systematic errors in chemical analyses (see chapter III) only the presence of an unknown cationic substance (when (cat min  $\text{Cl} + \text{HCO}_3^-$ ) is lower than expected) or anionic substance (for the values higher than expected) remains as an explanation.

It is possible that the solutions sometimes contained small amounts of  $\text{NH}_4^+$ -ions at the end of the dialysis, but it is unlikely that this ion can reach the concentration of  $2.3$  mEq/Kg  $\text{H}_2\text{O}$  necessary to explain the negative values for (cat min  $\text{Cl} + \text{HCO}_3^-$ ), which were occasionally observed. We could find no correlation whatsoever between the presence of *P. aeruginosa* in the dialysis solution and the phenomenon under discussion. This bacteria is not known to produce ammonium in any case. If the ammonium resulted from decomposition of proteins by plasma enzymes, this process should have to produce  $2.3$  millimoles of  $\text{NH}_4^+$ -ions from substrate of about  $4$  g of protein and  $20$  mg ( $= 0.3$  millimoles) of urea. This would mean that  $40$  millimoles of  $\text{NH}_3$  should be split off from  $100$  g protein, change which should have had considerable influence on NCE. The results of the experiments with stored serum or plasma argue against such an occurrence.

Possibly filtrable cationic substance was released from the walls of the containers. We have done sham experiments in which the containers were filled with  $\text{NaCl}$  solution or with dialysis solution only. The results of the experiments in which saline was used have already been presented for other reasons in chapter III in the section on  $\text{Cl}$  determination, but no evidence for the release of an unknown ion was found. Table 35 summarises the results of another experiment in which 2 containers were shaken with dialysis solution for 18 hours. In this experiment inorganic phosphate was determined too. In both containers the change in (cat min anions) was less than twice the SE of the mean.

We have as yet no explanation for the observed deviation of (cat min  $\text{Cl} + \text{HCO}_3^-$ ) from the value expected. But the fact that sign and magnitude of the deviation were always the same for dialysis solution and for distilled water suggests that the phenomenon will have no influence on our calculation of  $\Delta$  (cat min  $\text{Cl} + \text{HCO}_3^-$ ), since the latter is obtained from the difference between the value found inside and the one



found outside a dialysis unit. In chapter VII we will nevertheless consider separately the results obtained in the experiments in which (cat min Cl + HCO<sub>3</sub>) of the dialysis solution was within the probable range and in those in which (cat min Cl + HCO<sub>3</sub>) was clearly outside that range.

Apart from the question of their total concentration there is the question of the distribution of the anions not determined by us. It will be evident that  $\Delta$  (cat min Cl + HCO<sub>3</sub>) can only be interpreted as representing NCE if the concentration of all other anions is equal inside and outside the dialysis unit. In view of the effect of the Donnan equilibrium on the one hand and the possible existence of protein-bound rest anion on the other hand we will have to be more explicit on this point.

Because of the Donnan equilibrium one would expect the concentration of phosphate (expressed per Kg H<sub>2</sub>O) to be lower inside the serum-containing unit. However we already discussed in chapter IV the observations of others who found in dialysis or ultrafiltration experiments either an increased concentration at the protein-containing side of the semipermeable membrane (GREEN & POWER 1931; WALTER 1961) or an equal concentration on both sides (HOPKINS *et al.* 1952). SEENLES (1936) who worked with bovine serum could find no clear evidence of protein-bound phosphate. Our own results with *in vitro* ultrafiltration agree best with the observations of Hopkins *et al.* and indicate that at phosphate concentrations up to 10 mg / and under the conditions of *in vitro* ultrafiltration the effects of binding by protein and of the Donnan equilibrium cancel each other. Furthermore we observed in one dialysis experiment with normal dialysis solution no significant difference between the phosphate concentration inside and outside the protein-containing dialysis units (table 36). This table also includes the results of 3 experiments with a dialysis solution containing a fairly high concentration of phosphate buffer instead of the usual bicarbonate/CO<sub>2</sub> mixture. The solution contained normal concentrations of Na, K and Cl but no Ca or Mg. It can be seen that at these high concentrations the phosphate content on the inside was slightly lower than on the outside but the difference was not more than 4 %. Walser found that the percentage of inorganic phosphate presumably bound by protein is highest if the phosphate concentration is low. We can therefore conclude that at the concentrations used in our experiments there is no significant difference between the concentration of inorganic phosphate inside the serum-containing units and that outside them.



### Summary of chapter I

$\Delta$  Na,  $\Delta$  K,  $\Delta$  Ca and  $\Delta$  Ca + Mg could be determined with reasonable accuracy and expressed in mEq/100 g protein. However to obtain from those values the quantity of protein bound cation a correction must be made for the effect of the Donnan equilibrium as the latter causes the concentration of 'free' filtrable cations to be lower on the protein-containing side of the membrane than on the other side. For this correction the Donnan

ratio must be known. The latter was not determined directly but in chapter VIII an attempt will be made to estimate its value from circumstantial evidence.

The accurate determination of  $\Delta$  (cat min  $\text{Cl} + \text{HCO}_3$ ) is rather difficult in view of the cumulative effect of errors in determination and interpretation. But the available evidence indicates that in the case of the units containing at least 50 g/Kg  $\text{H}_2\text{O}$  protein the systematic error - if present - must have been small (less than 1 mEq/100 g protein) and must have led to overestimating rather than underestimating  $\Delta$  (cat min  $\text{Cl} + \text{HCO}_3$ ). The evidence further suggests that the latter is equal to the NCE of the proteins in the experiments in which serum was dialysed. When plasma was dialysed the heparin present may have made us overestimate the NCE of the proteins by about 0.5 mEq/100 g protein.

Quite apart from the theoretical difficulties involved, the distribution of the H-ions calculated from the pH measurements in our experiments could not be used for the determination of the Donnan ratio because of a small but systematic error in the determination of the pH of the solutions inside the dialysis units.

found outside a dialysis unit. In chapter VII we will nevertheless consider separately the results obtained in the experiments in which (cat min Cl + HCO<sub>3</sub>) of the dialysis solution was within the probable range and in those in which (cat min Cl + HCO<sub>3</sub>) was clearly outside that range.

Apart from the question of their total concentration there is the question of the distribution of the anions not determined by us. It will be evident that  $\Delta$  (cat min Cl + HCO<sub>3</sub>) can only be interpreted as representing NCE if the concentration of all other anions is equal inside and outside the dialysis unit. In view of the effect of the Donnan equilibrium on the one hand and the possible existence of protein bound rest anion on the other hand we will have to be more explicit on this point.

Because of the Donnan equilibrium one would expect the concentration of phosphate (expressed per kg H<sub>2</sub>O) to be lower inside the serum-containing unit. However we already discussed in chapter IV the observations of others who found in dialysis or ultrafiltration experiments either an increased concentration at the protein-containing side of the semipermeable membrane (GREEN & POWER 1931 WALSER 1961) or an equal concentration on both sides (HOPKINS *et al* 1952) SEEKLES (1936) who worked with bovine serum, could find no clear evidence of protein bound phosphate. Our own results with *in vivo* ultrafiltration agree best with the observations of Hopkins *et al* and indicate that at phosphate concentrations up to 10 mg / and under the conditions of *in vivo* ultrafiltration the effects of binding by protein and of the Donnan equilibrium cancel each other. Furthermore we observed in one dialysis experiment with normal dialysis solution no significant difference between the phosphate concentration inside and outside the protein-containing dialysis units (table 36). This table also includes the results of 3 experiments with a dialysis solution containing a fairly high concentration of phosphate buffer instead of the usual bicarbonate/CO<sub>2</sub> mixture. The solution contained normal concentrations of Na, K and Cl but no Ca or Mg. It can be seen that at these high concentrations the phosphate content on the inside was slightly lower than on the outside but the difference was not more than 4 %. Walser found that the percentage of inorganic phosphate presumably bound by protein is highest if the phosphate concentration is low. We can therefore conclude that at the concentrations used in our experiments there is no significant difference between the concentration of inorganic phosphate inside the serum-containing units and that outside them.



#### Summary of chapter V

$\Delta$  Na,  $\Delta$  K,  $\Delta$  Ca and  $\Delta$  Ca + Mg could be determined with reasonable accuracy and expressed in mEq/100 g protein. However to obtain from these values the quantity of protein bound cation a correction must be made for the effect of the Donnan equilibrium as the latter causes the concentration of free filtrable cations to be lower on the protein containing side of the membrane than on the other side. For this correction the Donnan

ratio must be known. The latter was not determined directly but in chapter VIII an attempt will be made to estimate its value from circumstantial evidence.

The accurate determination of  $\Delta$  (cat min  $\text{Cl} + \text{HCO}_3$ ) is rather difficult in view of the cumulative effect of errors in determination and interpretation. But the available evidence indicates that in the case of the units containing at least 50 g/kg  $\text{H}_2\text{O}$  protein the systematic error - if present - must have been small (less than 1 mEq/100 g protein) and must have led to overestimating rather than underestimating  $\Delta$  (cat min  $\text{Cl} + \text{HCO}_3$ ). The evidence further suggests that the latter is equal to the NCE of the proteins in the experiments in which serum was dialysed. When plasma was dialysed the heparin present may have made us overestimate the NCE of the proteins by about 0.5 mEq/100 g protein.

Quite apart from the theoretical difficulties involved, the distribution of the H-ions calculated from the pH measurements in our experiments could not be used for the determination of the Donnan ratio because of a small but systematic error in the determination of the pH of the solutions inside the dialysis units.



### PART III



*Results obtained with in vivo ultrafiltration  
(including normal values for the plasma ions)*

Both normal persons and patients have been studied. The results obtained in normal persons will be presented in some detail with due regard to the systematic errors. The results obtained in patients are less accurate since both the number of samples and the percentage increase in protein were smaller than in the normal subjects. But in the case of the patients the results will at least indicate the magnitude of the changes to be expected in the diseases studied.

### NORMAL SUBJECTS

#### DATA CONCERNING SUBJECTS STUDIED AND PRESENTATION OF RESULTS

28 male subjects were studied, all members of our medical staff or senior medical students, varying in age from 23 to 44 years. Some subjects were studied more than once so that the total number of investigations in normal persons was 34. The investigations were done in the course of 1½ year but the conditions of the investigation proper remained constant (see chapter IV).

The results can be described under three headings

- (1) the plasma concentrations of Na, K, Ca, Mg, Cl and  $\text{HCO}_3$  in the fasting and resting subject (venous blood, obtained without stasis)
- (2) values found for free and protein-bound Na, Ca and Mg
- (3) values found for RA and for NCE

In table 37 the results of the individual experiments are presented. The values were obtained in the manner described in chapter IV and in table 37 they are not yet corrected for the systematic errors discussed in chapter III and IV. An exception to this are the last columns which give for RA, NCE, free and protein-bound Ca the values corrected for the error resulting from pH change during venous compression. For this error a correction can be made in the individual experiment. It should be noted that in table 37 the values for NCE and for protein-bound cation are expressed per 100 g of plasma protein. The actual values in plasma can be calculated with the aid of the protein concentration of the precompression samples.

Table 37 further includes data pertinent to the calculation or the interpretation of the results. For each investigation the equation used for calculating water content is given (between brackets the number of samples in which water content was actually determined). Pre- and endcompression pH are listed to indicate the pH change during compression. Protein fractions are given when determined. Furthermore, since both the number of samples and the degree of haemoconcentration



tration affect the accuracy of the regression lines, the total number of samples and the percentage increase in protein concentration (g/Kg H<sub>2</sub>O) are mentioned for each investigation.

In figg. 40a 40b and 40c we have for each experiment graphically represented the data from which NCE and RA were calculated. The manner of presentation is the same as in fig 20 (chapter IV). The precompression, compression, and postcompression samples are indicated by different symbols.

The graphs will enable the reader to get an idea of the significance of the individual experiments. Obviously less accurate values will be obtained for NCE and RA from experiments with a relatively small number of samples and/or a relatively small increase in protein concentration. Statistically therefore the experiments must have a different "weight" and this should have its effect on the calculation of the mean value. However in the Addendum it will be shown that the mean values calculated for NCE and for RA with due regard to the differences in the "weight" of the results of the individual experiments, differ not more than 0.2 mEq/100 g protein, resp 0.2 mEq/Kg H<sub>2</sub>O from the mean values calculated by simply averaging the values obtained in the individual experiments. In subsequent calculations we will therefore regard the individual results as having equal "weight" statistically.

We have refrained from graphically reproducing the individual experiments for Na, for Ca and for Ca + Mg both for reasons of space and because for these cations the scatter of the experimental points around the calculated regression line is much smaller than for NCE (VAN LEEUWEN *et al* 1961).

Since for systematic errors other than those due to the pH change during compression only the direction and the average magnitude can be estimated and their cumulative effect remains relatively small (an exception must be made in the case of protein-bound Na), we will postpone the correction for the systematic errors till after a consideration of some aspects of the experimental results proper.

#### DISCUSSION OF EXPERIMENTAL RESULTS PROPER

##### Water content of samples - Relation with protein content

As mentioned in chapter IV concentrations were converted from mEq/L plasma to mEq/Kg H<sub>2</sub>O in the following manner. For at least one pre- and one endcompression sample water content was determined (chapter III). On the assumption that the relation between protein content (g/L plasma) and water content (g/L plasma) is linear for the samples of one investigation we calculated for each investigation the constants *a* and *b* in the following equation

$$\text{Water (g/L plasma)} = a - b \times \text{protein (g/L plasma)}$$

In 31 of the 34 investigations reported in table 37 water content was determined in at least two samples which differed more than 25 g/L in protein concentration. In table 37 it can be seen that in these experiments the value found for *a* varied from 992.5 to 1007.5 and the value found for *b* from 0.754 to 0.999.

Averaging the results for all investigations we found

$$\text{Water (g/L plasma)} = 996.1 - 0.828 P \text{ (g/L plasma)}$$

In three cases (Gee, dVr I and Dou) the values found for *a* and *b* were considerably outside the rather narrow range found for the other experiments. We have no explanation

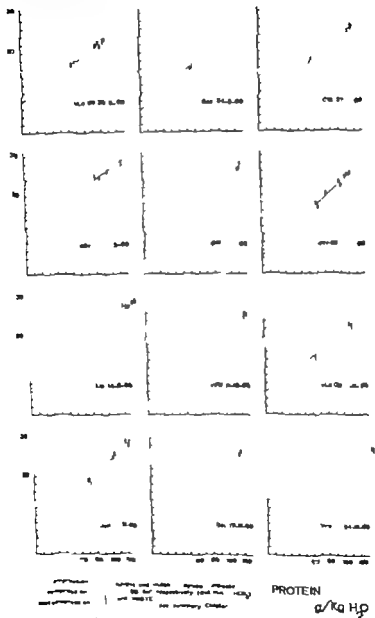
$\text{Ca HCO}_3 \quad \text{mEq/Kg H}_2\text{O}$ 

Fig 40c Graphs representation of the 34 *in vivo* ultrafiltration experiments in normal subjects. The diagonal lines represent the regression lines from which  $\rho_{CE}$  and  $\rho_{IA}$  were obtained (see table 37).

CATIONS  
minus  
CL+HCO<sub>3</sub> mEq/Kg H<sub>2</sub>O

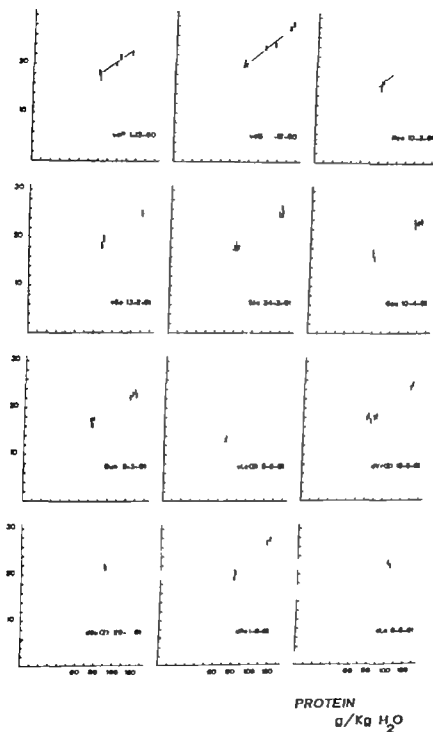


Fig. 40b - See caption and legend of fig. 40a.

CATIONS

minus

CL  $\text{HCO}_3$  mEq/Kg  $\text{H}_2\text{O}$

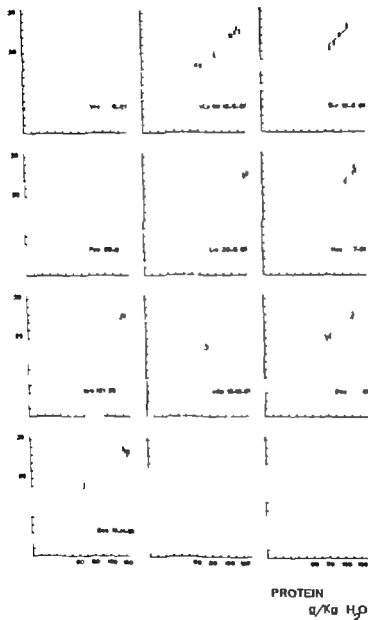


Fig. 40c: See caption and legend of Fig. 40a.

for this excentricity the triplicate determinations showed good agreement. But if we leave out the three excentric observations we calculate the following average equation for normal plasma

$$W = 995.3 - 0.814 P$$

The equations indicate a specific volume of about 0.82 for the plasma proteins.

In the cases where more than two samples with different protein concentration were dried the experimental points always did lie on a straight line

For each investigation we also calculated the correlation between dry weight and protein concentration (both in g/L plasma) again assuming linearity. The dry weight at zero protein concentration represents the weight of all plasma constituents other than protein in as far as they are not bound in one way or other to protein. For all 31 investigations together the average dry weight at protein concentration zero was thus calculated at 9.1 g/L plasma, and when the three excentrics are left out at 9.9 g/L plasma. Since roughly 10% of Na and of K and about 40% of Ca and Mg are 'bound' by serum proteins (GERBRANDY *et al.* 1960) we can calculate the dry weight of the remaining cations and anions at 8.5 g/L plasma. In the fasting subject glucose is less than 0.9 g/L and urea about 0.3 g/L. Uric acid, aminoacids and creatinine together should be less than 1 g/L. The lipids present in plasma from a fasting subject are completely bound to proteins and will not contribute to the dry weight at zero protein concentrations. There is therefore a reasonable agreement between the expected dry weight and the value found by extrapolation from our experimental values.

In the 18 dialysis experiments performed with serum of normal subjects (practically all of whom also partook in the *in vivo* ultrafiltration study) we found the following average equation

$$\text{Water (g/L plasma)} = 996.0 - 0.815 P \text{ (g/L plasma)}$$

In the individual experiments the value of *a* varied between 995.5 and 997.7 and that of *b* between 0.763 and 0.855. In these experiments the relation was calculated from the dry weight of the dialysis solution and that of one or more of the serum samples.

We have preferred to use the correlation found in the individual experiment instead of the average equation for calculating concentrations per Kg H<sub>2</sub>O because in the case of patients only the individual correlation can be used: some nephrotics have a rather high specific volume of the proteins presumably because of the secondary hypercholesterolaemia observed in these cases. In the uraemics the value of the extrapolated dry weight was occasionally as high as 16 g/L.

Although the use of the individual correlation instead of the average equation must somewhat increase the spread in the values obtained for protein bound cation the effect is negligible in the case of NCE since both cations and anions are multiplied with the same factor. Even in the three cases where the correlation between water content and protein concentration was well outside the range observed in the other normal subjects, the values of NCE obtained from molal concentrations calculated either by means of the individual correlation or by means of the average equation found for normal subjects differed less than 0.1 mEq/100 g protein. In the three *in vivo* experiments and two dialysis experiments in which no water content was determined the general equation 996-0.82 P was used.

A comparison of our values for plasma water with those found by others must wait till another occasion. Also for reasons of space, we shall not discuss the water content found in the case of the patients or in the case of the dialysis experiments.

The correlation between (cat min Cl +  $\text{HCO}_3$ ) and protein concentration as observed in the individual experiments

For this we must consider the experiments in which the protein concentration of the various compression samples shows a gradual rise resulting in a uniform distribution of the experimental points along the regression line (figg. 40a 40b 40c). In most of these experiments the linearity is evident but in some (dVr 18-5-61 Poo 28-6-61 and vOp 16-10-61) the points representing the first compression samples clearly fall below the regression line.

This dip was not seen in the correlation between Na, Ca or Ca + Mg and protein concentration. In the three experiments mentioned above the low values for (cat min Cl +  $\text{HCO}_3$ ) in the first compression samples resulted from the fact that the  $\text{HCO}_3$  concentration increased immediately after starting compression whereas the Cl concentration decreased more gradually. We have not been able to explain this phenomenon. A relative increase in  $\gamma$ -globulin which would decrease NCE was never observed nor sudden decrease in pH, in lactate or in inorganic phosphate concentration.

But even if this deviation from linearity is real its effect on the mean values calculated for NCE and RA in normal subjects must have been small. This can be demonstrated by calculating, for each of the experiments in which the points representing the compression samples are distributed in a uniform manner over the protein range, the regression line for the compression points only. The slope of the regression line thus calculated will largely depend on the points representing the first samples taken after the start of compression, since they determine the position at its lower end. A slight downward deviation of these points will cause the slope of the regression line calculated for the compression points to be much steeper than that of the regression line calculated for all points. Table 38 demonstrates that there is indeed a difference between the mean values of NCE and of RA calculated from all samples of an experiment (first 2 columns), and the values calculated from the compression samples only (last 2 columns), but that the difference is a small one. Furthermore since the number of points available for the calculation of the second regression line is rather small, especially at the critical lower end of the line, the accuracy of the values in the last 2 columns is less than that of the values in the first 2 columns and we have therefore concluded that in our experiments the correlation between (cat min Cl +  $\text{HCO}_3$ ) and protein concentration is on the average sufficiently linear to allow the calculation of one regression line for all the samples of an experiment. The following observations appear to endorse this conclusion. In 5 experiments the first postcompression sample was taken within two minutes after release of the pressure and therefore still has a rather high protein concentration. The value found for (cat min Cl +  $\text{HCO}_3$ ) in these samples fits remarkably well into the values found for the compression samples. There is thus no sign of hysteresis and a similar conclusion can be drawn from the finding that pre- and postcompression samples show on the average nearly the same values for (cat min Cl +  $\text{HCO}_3$ ). (See table 27 in chapter IV)

Correlation between RA and NCE and between 'free cation and protein-bound cation

Theoretically no correlation should exist between the values calculated for RA and for NCE, whereas one would expect a positive correlation between the concentration of 'free cation and the amount of protein-bound cation. In fig. 41 we have plotted the

values given in table 37 for NCE respectively protein bound cation against the corresponding values for RA respectively free cation (the values are those not corrected for any systematic error). It will be seen that in every instance a negative correlation was found. The obvious explanation is that RA respectively free cation concentration is not determined independently but is obtained by extrapolating the regression line to protein concentration zero. The normal scatter in the slope of the calculated regression line will therefore result in an inverse scatter of the values found for RA respectively free cation. From fig. 41 it is evident that within the normal range this scatter effect overrules the fundamental physico-chemical relationship. This observation serves to stress the fact that for RA and for free cation the average of the individual values rather than the individual values themselves should be considered.

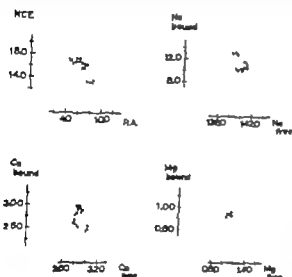


Fig. 41 - Correlation between RA and NCE, respectively free and protein-bound cation as observed in the normal subjects (RA and free cation in mEq/kg H<sub>2</sub>O, NCE and protein-bound cation in mEq/100 g protein).

### Changes in normal values observed in the course of one and a half year

In fig. 42 the values given in table 37 for plasma concentration and for NCE and protein bound cation (not corrected for any systematic error) have been plotted against the date of the investigation. It can be seen that the values show a reasonably equal distribution around the lines representing the mean values calculated from the first 28 experiments in table 37. Fig. 42 also demonstrates that the values found on repeated investigation of the same subject show a spread similar to that found for the group of normal subjects considered as a whole at least for NCE and protein-bound cation.

### CORRECTION FOR SYSTEMATIC ERRORS

In chapter III and IV we discussed a number of systematic errors for which the results given in table 37 should be corrected. For all but one of the errors only the average effect can be estimated and this with a varying degree of certainty. In the following paragraphs the magnitude and direction of these errors are considered (for argumentation the reader is referred to chapter III and IV).

The magnitude of the four errors to be considered first can be estimated with a fair degree of certainty but in the case of the last two the estimate is much more dubious.

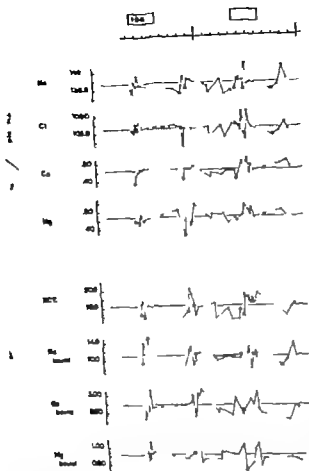


Fig. 42 Relation between date of experiments and values for normal subjects. The symbols  $\alpha$ ,  $\lambda$ ,  $\Delta$  and  $\square$  indicate the four persons who were studied more than once.

(a) *Effect of the heparin solution added.* This will depend on the amount and the composition of the heparin solution added and on the plasma volume of the blood sample. For the samples of one experiment the first two are constant but the latter varied according to hematocrit and total volume of the blood sample. Since the hematocrit of both pre- and an endcompression sample was determined in 17 of the experiments in table 37 (numbers 1, 16 and 29), we can use the results of these experiments for calculating the magnitude of the heparin error in the following manner. When the amount of heparin solution ( $V_{\text{hep}}$ ) and its composition ( $C_{\text{hep}}$ ) are known as well as the combined volume of the plasma-heparin mixture ( $V_{\text{pl+hep}}$ ) and its composition ( $C_{\text{pl+hep}}$ ) the concentration in the original plasma ( $C_{\text{pl}}$ ) can be calculated from

$$C_{\text{pl}} V_{\text{pl}} = (C_{\text{pl+hep}} V_{\text{pl+hep}}) - (C_{\text{hep}} V_{\text{hep}}).$$

In the 17 experiments, referred to above, the average value of the hematocrit was 44 for the precompression and 57 for the endcompression samples whereas the sample volumes were on the average 16 and 17 ml respectively. The composition of the heparin solution is known (see chapter III) and with the aid of these data and of the equation mentioned above we could make an estimate of the resulting errors.

In the case of protein, Ca, Mg and  $\text{HCO}_3$  the concentration decreases by 0.3 respectively 0.3 as result of adding 0.01 respectively 0.03 ml heparin solution. These errors are so small, both



absolute and relative, that we have neglected them. Since the Cl and K concentration of the heparin solution are of the order of the plasma concentration of these ions no error is made in their case. For Na the concentration increases by about 0.1 respectively 0.3 / when 0.01 respectively 0.03 ml heparin solution are added. In this case the absolute error is not negligible and owing to the fact that the error was somewhat larger in the end compression samples (where the plasma volume was smaller than in the precompression samples) protein-bound Na is also somewhat overestimated. As a result NCE and RA are overestimated as well. In table 19 the magnitude of these errors is shown (the error given for protein-bound Na and for NCE includes the error due to dilution of the protein concentration).

As during the first part of our study 0.01 ml heparin solution was added to the samples but later 0.03 ml the mean values of the corresponding episodes were corrected separately.

(b) Flame photometer error in determination of Na and K. In chapter III we concluded that both Na and K concentrations are underestimated by on the average 0.1 mEq/L at a protein concentration of 60 g/L and 0.2 mEq/L at a protein concentration of 120 g/L. The values obtained on precompression samples are presumably therefore for both cations on the average 0.1 mEq/L too low. Furthermore, as a result of the extrapolation the amount of protein-bound Na will be underestimated by  $100/60 \times 0.1 = 0.15$  mEq per 100 g protein, and NCE (to which both Na and K contribute) by double this amount i.e. 0.3 mEq per 100 g protein. The respective average values must be corrected accordingly. The error has no influence on the calculation of the amount of free Na and K and of RA.

(c) Error caused by change in pH during compression of upper arm. As both the pH and the protein changes were determined in each experiment it is possible to correct the individual results in the case of NCE and of protein-bound Ca, because data exist concerning the change of these quantities with pH (chapter IV). The corrected values thus obtained are listed in the last columns of table 37. For protein-bound Na and Mg only an approximate correction of the average values can be made.

(d) Error caused by measuring blood pH at 37.5 °C. As stated in chapter IV the temperature of the venous blood must have about 35.5 °C on the average. The resulting error in pH measurements - an underestimation of 0.03 - causes  $\text{HCO}_3^-$  to be underestimated by 0.1 mEq/Kg  $\text{H}_2\text{O}$  in the mean. But this only influences the calculation of RA, the average value of which should accordingly be corrected by subtracting 0.1 mEq/Kg  $\text{H}_2\text{O}$ . The pH to which the values calculated for protein-bound cation and for NCE must be referred is the average value of the endcompression pH (see chapter IV) with 0.03 added for the temperature error. Similarly to obtain the pH of venous plasma in the fasting and resting subject, 0.03 must be added to the average precompression pH value.

(e) Error due to neglect of the presence of carbonate and of carbamino-protein. In chapter III reasons were given for assuming the combined effect to be an overestimate of  $\text{HCO}_3^-$  by 0.1 mEq/L at pH 7.40 and by 0.2 mEq/L at pH 7.80 in otherwise normal plasma. Because of a linear correlation with protein concentration the error due to carbamino-protein was estimated to increase from + 0.2 to + 0.4 mEq/L, if the protein concentration increases from 60 to 120 g/L. Accordingly NCE will be underestimated by  $100/60 \times 0.2 = 0.3$  mEq/100 g protein and the value of RA will be overestimated by 0.1 mEq/Kg  $\text{H}_2\text{O}$ . These values should apply when pH is about 7.40.

(f) Error due to the effect of the Donnan equilibrium. In chapter IV it was concluded that the calculation of NCE is not affected by this error but that the calculation of protein-bound Na, Ca and Mg is. In chapter IV we have estimated the error to be an underestimation of 3-4 mEq/100 g protein in the case of Na, and an underestimation of 0.1-0.2 mEq/100 g protein in the case of Ca. Since in the case of Mg about the same percentage of the total cation concentration is protein-bound as in the case of Ca, and presumably in the same manner it is reasonable to assume the same percentage underestimation of protein-bound Mg i.e. about 0.05 mEq/100 g protein. Since the error due to the Donnan equilibrium is linearly related to the protein concentration it has no effect on the calculation of the free fractions at zero protein concentration. But the actual protein concentration of the precompression samples the concentration of free cation must have been about 3 mEq/Kg  $\text{H}_2\text{O}$  lower for Na and about 0.1 mEq/Kg  $\text{H}_2\text{O}$  lower for Ca.

## RESULTS CORRECTED FOR SYSTEMATIC ERRORS

In the following tables the experimental values given in table 37 are corrected for the errors mentioned and a best estimate of the average values is made.

Table 40 summarises the (venous) plasma concentration (mEq/L) of Na K, Ca Mg Cl and  $\text{HCO}_3$  in the fasting and resting subject (blood obtained without stasis). In this table also the protein concentration and composition are given and the concentration found for inorganic phosphate

In table 41 the values for free (mEq/Kg  $\text{H}_2\text{O}$ ) respectively protein-bound (mEq/100 g protein) Na, Ca and Mg are given.

Table 42 finally presents the values for RA (mEq/Kg  $\text{H}_2\text{O}$ ) and for NCE (mEq/100 g protein).

It is evident that the total error for RA and for NCE is largely determined by the error caused by the change in pH during venous compression

## ADDITIONAL OBSERVATIONS

Correlation between endcompression pH respectively protein composition and protein-bound cation and NCE

As both pH and protein composition affect the amount of cation bound by protein it is of some interest to see whether this effect is detectable within the range of normal values. To that purpose fig. 43 was constructed.

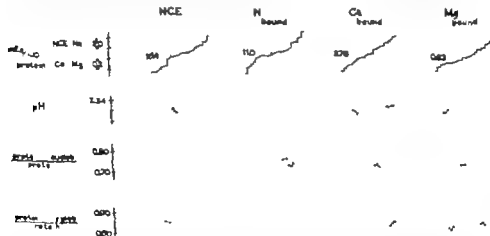


Fig. 43 Correlation between endcompression pH, respectively protein composition, and NCE and protein-bound cations (normal subjects)

We have arranged the individual values presented in table 37 according to the magnitude of NCE, respectively protein-bound cation, starting with the lowest also. Below these values the concomitant endcompression pH and protein composition were plotted. The latter is indicated by the ratio (protein  $\gamma$ -glob)/protein respectively the ratio (protein  $\gamma$ -glob)/protein according to whether the method of Major or paper electrophoresis. We have chosen these ratios because the evidence available at present suggests that albumin,  $\alpha$ -globulin and  $\beta$ -globulin have the highest cation-binding capacity and  $\gamma$ -globulin the lowest.

Fig 43 shows that only between protein bound Ca and protein composition some correlation appears to exist. Furthermore we found that in the case of NCE and of protein bound Ca it makes no difference whether the experimental values proper or the values corrected for the effect of pH change are considered. The correction does not affect the spread nor the distribution of the individual values around the mean value.

#### Comparison of the values obtained for protein-bound Ca and Mg with the values obtained in earlier experiments

We have previously published values for free and protein bound Ca and Mg obtained with the same method (VAN LEEUWEN *et al* 1961). In table 43 the results of the earlier study are compared with the present results. Since in the first study no correction was made for systematic error the results of the present study are also given without correction. The two series have only one experiment in common (Lie 14-6-60) but 12 of the subjects figure in both series. From table 43 it would appear that the values found for protein-bound cation in the present study are about 6% lower than those found in our previous study. The difference is due to the fact that the protein concentration obtained in the first study was afterwards found to be 3% too low up to a protein concentration of about 70 g/L. The error became increasingly larger for protein concentrations higher than 70 g/L. The error was due both to an error in the Kjeldahl determination and to an error in the calibration line used with the biuret method (see chapter III). Because of this the amount of cation bound per 100 g protein was in the earlier experiments overestimated by about 5% and - as a result of extrapolation - the concentration of free cation was underestimated by about 2%. This fully explains the difference between the results of the two studies.

#### Results obtained when Na and K are analysed in deproteinised plasma

In a number of experiments presented in table 37 Na and K were determined both in plasma and in deproteinised plasma since at the time we could not yet decide which of the two techniques was the more accurate one. However during that period 6 investigations on patients were done in which only deproteinised plasma was analysed. These observations cannot be compared with the results obtained for the other patients unless a correction is made. To provide the latter table 44 has been added which shows the values obtained when plasma and the values obtained when deproteinised plasma was analysed in 14 experiments with normal subjects. As the difference between the Na concentration obtained with plasma and that obtained with deproteinised plasma increases with increasing protein concentration (see chapter III) not only a higher value for RA is found with deproteinised plasma but also a higher value for NCE. On the average the difference was 1.3 mEq/Kg  $H_2O$  for RA and 1.9 mEq/100 g protein for NCE.

## PATIENTS

### DATA CONCERNING SUBJECTS STUDIED AND PRESENTATION OF RESULTS

29 patients of both sexes (age 25 to 87 yr) were studied in the manner described in chapter IV. The diseases from which they suffered can be classified in two groups:

- (1) Diseases mainly characterised by an abnormality in protein composition either a

quantitative disproportion between the normal fractions (which shall be indicated by the term dysproteinæmia) or the presence of an abnormal protein (indicated as para proteinæmia).

(2) Diseases mainly characterised by an abnormality in the other plasma constituents. The patients with severe chronic uræmia are grouped together. The others - including patients with hypercalcaemia and hypercholesterolaemia - are listed under miscellaneous together with one apparently normal reconvalescent.

The conditions of the actual investigation were the same as for the normal persons, but in 6 patients Na and K concentration were determined on deproteinised plasma only.

The results of the individual experiments are compiled in table 45. The lay-out of the table differs somewhat from that of table 37 and needs some explanation.

The results have been grouped according to the main abnormality in the plasma but overlapping could not be avoided.

In table 45 the Hb concentration has also been listed. In the presence of severe anaemia venous congestion tends to raise venous blood lactate (chapter IV) and neglect of this would result in the calculation of too high value for NCE and too low value for RA. Consequently special caution is necessary in the interpretation of these values when Hb is low.

When in the column headed 'other substances' creatinine is not mentioned the glomerular filtration was at least 70 ml/min.

In the last four columns are listed the values found for RA, NCE, free and protein-bound Ca after correction for the error caused by the pH change during compression. In the case of the patients this correction was sometimes rather large because, although the pH change was of the same order as in the normal subjects, the percentage rise in protein concentration was considerably smaller in most patients.

At the end of each subgroup we have listed the results of the investigations in which Na and K were only determined on deproteinised plasma. Both the plasma concentration of these substances and the values calculated for RA and NCE, respectively free and protein-bound Na are too high. These values have therefore been marked with an asterisk.

For normal subjects the results obtained with deproteinised plasma are on the average 1.3 higher for RA (mEq/Kg H<sub>2</sub>O) and 1.9 higher for NCE (mEq/100 g protein) (table 44). But for the 6 investigations of patients, in which flame photometry was performed both on plasma and on deproteinised plasma, the values calculated from the latter were on the average 2.1 higher for RA and 1.0 higher for NCE (table 46). The difference between the average values in table 44 and the corresponding average in table 46 is presumably due to the fact that the protein concentration was less in the patients. We have therefore concluded that the values which would have been obtained if plasma had been analysed, can be estimated by subtracting from the values actually obtained with deproteinised plasma 1.5 for RA (mEq/Kg H<sub>2</sub>O) and 1.5 for NCE (mEq/100 g protein). This correction should increase the accuracy of the individual results by not more than 2 mEq/litre either RA or NCE (see table 44 and 46).

## COMPARISON WITH THE RESULTS OBTAINED IN NORMAL SUBJECTS

### NCE and RA

In fig. 44 the results obtained in patients are compared with the values found for normal subjects. Disease can alter the protein concentration of plasma, the protein composition or both. We have therefore compared the actual values of NCE (mEq/l. plasma) as well as the values obtained when NCE is expressed per unit weight of protein (mEq/100 g). The first values indicate the effect of the disease on the NCE of plasma, the latter give information concerning changes in the characteristics of the proteins themselves.

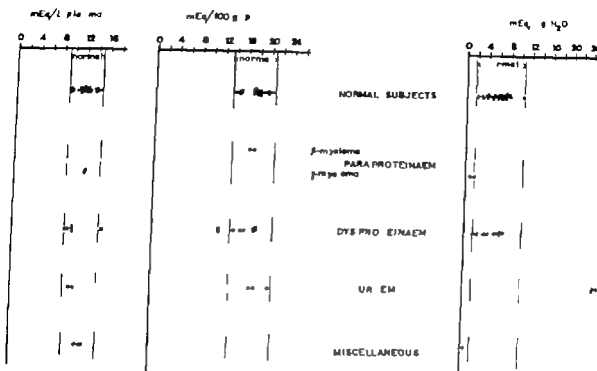


Fig. 44 - Comparison of results obtained for patients with those obtained for normal subjects. For explanation see text.

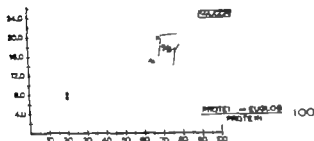
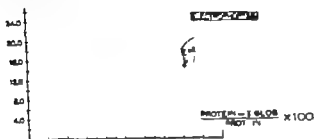
All points in fig. 44 refer to the values obtained after correction for the effect of pH change during venous compression. The values from experiments in which Na and K were determined on deproteinized plasma are indicated by open circles. They were obtained by subtracting 1.5 from those values listed for RA and for NCE in the last four columns of table 45 which are indicated by an asterisk. Finally the actual plasma values for NCE were calculated from the (corrected) values per 100 g protein and the protein concentration of the precompression samples (also listed in table 45).

Somewhat to our surprise we found NCE in the normal range in most of the patients we studied, even in those who were severely uraemic. Indeed, if we take into account the larger spread to be expected in the case of the patients, we must conclude that only in the patients with paraproteinaemia abnormal values were found. For the patients with  $\gamma$  myeloma the values are definitely too low when expressed per 100 g protein but when expressed per litre plasma they are nearly normal because of the high protein concentration in plasma. On the other hand in the 2 patients with  $\beta$ -myeloma NCE expressed per 100 g protein was normal, but expressed per litre plasma NCE was somewhat too high, again because of the increased total protein concentration. Evidently the paraproteins of the  $\gamma$ -globulin type have a very low NCE whereas those of the  $\mu$ -type appear to have a NCE comparable to that of albumin. The results, obtained in the case of  $\gamma$  myeloma, are in agreement with the findings of Gutman *et al* discussed in chapter II.

For RA abnormal values were only found in the case of the uraemic patients. In this

group there is a positive correlation between the value found for RA and the concentration of inorganic phosphate, the latter accounting for about 30% of the former (table 45). The same ratio was found in normal subjects but whether this observation has any meaning cannot be decided on this small number of investigations.

NCE corrected  
mEq/100 protein



PROTEIN

normal range  
β-thymoma  
β-myeloma

dysproteinemia  
albumin  
myeloma

Fig. 45 Correlation between composition of plasma protein and NCE (patients)

**Effect of protein composition on NCE** An attempt to correlate NCE (expressed in mEq/100 g protein) with protein composition meets with the difficulty that there are more than two protein fractions. And since we analysed plasma, fibrinogen was also present, which in the patients occasionally constituted as much as 20% of total protein. But the following reasoning appears to provide a means for analysing the effect of protein composition. From the dialysis experiments to be discussed in the next chapter we must conclude that both for normal and for abnormal  $\gamma$ -globulin NCE is practically zero at normal plasma pH. Consequently NCE should show a positive correlation with (total protein -  $\gamma$ -globulin) expressed as percentage of total protein. Fig. 45 appears to confirm this but the number of observations with a low value for (total protein -  $\gamma$ -globulin) is very small and fig. 45 does no more than indicate a certain trend.

As regards the relative contributions of albumin and of  $\alpha$  and  $\beta$ -globulin and fibrinogen to the value of NCE the following conclusions can be drawn from table 45. In the group of dysproteinemias there are 2 patients with a low albumin and a low  $\gamma$

globulin content (Ben and Ber) and in these cases NCE was therefore largely determined by the other protein fractions in both cases a normal value for NCE was found. This suggests that for  $\alpha$   $\beta$ -globulin and fibrinogen together NCE (in mEq/100 g protein) is of the same order as for albumin. Furthermore, for the patients with dysproteinaemia the average value of NCE was of the same magnitude as for the patients with uraemia and for the patients listed under miscellaneous. Since in the dysproteinaemia group the percentage albumin was on the average about half that found in the two other groups whereas the percentage  $\gamma$ -globulin was higher we are tempted to postulate that - per unit weight -  $\alpha$   $\beta$ -globulin and fibrinogen together have a higher NCE than albumin.

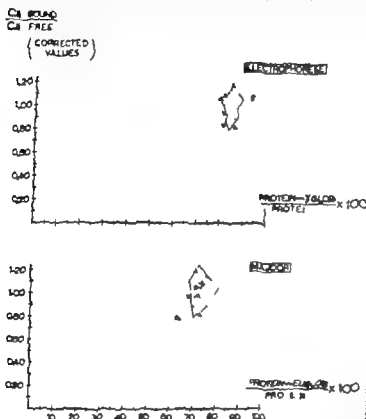


Fig. 46 Correlation between composition of plasma proteins and the ratio protein-bound Ca/free Ca (patients). See text for explanation.

**Effect of pH on NCE** Until now we have neglected the influence of the pH. Differences in the latter must have contributed to the spread in the values obtained. The extremes in endcompression pH observed in the patients were 0.25 pH unit apart and therefore purely because of the pH effect, the value of NCE should for these two patients differ by about 3 mEq/100 g protein if we accept the buffer value found by VAN SLIKE *et al* in 1928. However in most investigations the endcompression pH was in the same range as that found for the normal subjects. The 4 groups of patients differ somewhat in this respect the average endcompression pH being slightly higher for the patients with paraproteinaemia and somewhat lower for the patients with uraemia. This in itself should cause NCE to be overestimated in the first group and to be underestimated in the uraemic patients, but the difference between the pH values is so small that a corresponding correction of the average NCE values did not visibly change the relation between the 4 groups depicted in figs. 44 and 45.

### Free and protein-bound Na, Ca and Mg

For all three cations the amount bound per 100 g protein is lowest in the patients with paraproteinaemia and in the case of Na the difference between the  $\beta$ - and the  $\gamma$ -type of myeloma is evident. The difference is not so evident for Ca and in the case of Mg no difference can be detected. But especially for Ca the 'free' concentration varied considerably more in the patients than in the normal subjects and for this a correction should be made. To that purpose we have calculated for each experiment the ratio between protein-bound Ca (mEq/100 g protein) and 'free' Ca (mEq/Kg H<sub>2</sub>O), using the values obtained after correction for the error caused by the pH change during compression. This ratio should be constant if no change occurs in protein composition or in pH (MCLEAN & HASTINGS 1935<sup>1</sup>). In fig. 46 we have plotted this ratio against the ratio (total protein -  $\gamma$ -globulin)/total protein, and it can be seen that roughly the same correlation exists as for NCE: the abnormal  $\gamma$ -globulins bind considerably less Ca than the other fractions, but albumin and the other globulins apparently have about the same tendency to bind Ca, at least at normal pH values.

For Mg a similar attempt would be wasted because the spread in individual values is too large in comparison with the correlation to be detected. And since in the case of protein-bound Na the correction for the effect of the Donnan equilibrium is about 30 % of the uncorrected value we have also refrained from considering the correlation between protein composition and protein-bound Na in more detail.



## *Results obtained with equilibrium dialysis*

Most experiments were done with serum, a smaller number with solutions of albumin and  $\gamma$  globulin. The few experiments in which plasma was dialysed will be presented together with the results obtained with serum of normal subjects

### SERUM FROM NORMAL SUBJECTS

#### DATA CONCERNING SUBJECTS STUDIED - PRESENTATION OF RESULTS

23 dialysis experiments were done between November 1960 and February 1962. In 5 experiments plasma was dialysed, in 17 serum and in 1 experiment both plasma and serum. In 19 experiments the serum or plasma was obtained from one (fasting) subject but in 4 experiments pooled serum was studied each sample containing serum from 3 (non-fasting) subjects. The samples were dialysed directly after they were obtained. Since some subjects contributed more than once the total number of persons whose serum or plasma was studied is 19 one a female. All but 3 also underwent *in vivo* ultrafiltration and in most cases the sample used for dialysis was obtained immediately after the conclusion of the *in vivo* ultrafiltration experiment.

Nearly all serum samples were dialysed at three different pH values. The latter ranged from 6.33 to 7.96 but in all but 3 of the experiments the pH was within the range 6.60 to 7.80.

For the description of the method and the calculations involved the reader is referred to chapter V.

The results of the individual experiments (no correction for systematic errors) are given in table 47. For reasons of economy the small number of experiments in which pathological serum was dialysed have been listed at the end of table 47. They will be discussed separately. Table 47 needs some explanation.

The heading 'distilled water' refers to the solution inside the control unit after dialysis, the heading 'dialysis solution' to the composition of the solution outside the dialysis units at the end of the dialysis. Under the heading 'protein solution' are indicated respectively the number of dialysis units filled from the same serum (plasma) sample, whether the protein solution was plasma or serum, the mean pH value, the mean protein concentration and the mean values for NCE,  $\Delta$  Na,  $\Delta$  K and  $\Delta$  Ca + Mg for the number of units indicated (the maximum difference in protein concentration between the units filled from one sample was never more than 7 g per Kg H<sub>2</sub>O). For reasons discussed in chapter V only the results from units containing solution with more than 50 g/Kg H<sub>2</sub>O protein have been included in table 47. As to the protein composition the values placed between brackets are the values found for plasma in the preceding *in vivo* ultra-

tration experiment. In most cases serum was dialysed but the relative percentages of albumin and the different globulins must have been practically the same as for plasma.

Finally in the column containing the data concerning the concentration ratios the heading 'distilled water' refers to the distribution across the membrane of the control unit, the heading 'protein solution' to the distribution across the membrane of the serum or plasma containing units. In the latter case all ratios refer to a protein concentration of 20 g/Kg  $H_2O$  the reason for this will be discussed in the next section.

For Ca and Mg no separate results are given. In order to reduce the amount of analytical work only the sum concentration  $Ca + Mg$  was determined in all experiments but 4 and in these plasma was studied. The values obtained for  $\Delta Ca$  and  $\Delta Mg$  in these 4 experiments will be presented separately.

Concerning the interpretation of changes in  $Ca + Mg$  the following can be said. Both CAJUR (1955) and VAN ORSSE & KONPMAN - VAN EUPEN (1957) observed that albumin attracts Ca and Mg-ions apparently with equal strength. Presumably therefore a given change in pH will induce the same relative changes in albumin-bound Ca and in albumin-bound Mg. Although in serum the proteins bind a higher percentage of Ca than of Mg the difference is not large (see chapter VI) and in our dialysis experiments we can presumably interpret changes in the serum concentration of  $Ca + Mg$  as indicating equal (relative) changes in the concentration of each of the two cations.

#### SOME POINTS CONCERNING EXPERIMENTAL RESULTS AND THEIR PRESENTATION

Correlation between the values found for (cat min  $Cl + HCO_3$ ) in the dialysis solution and the values calculated for  $\Delta$  (cat min  $Cl + HCO_3$ ) i.e. NCE

In chapter V it was pointed out that in a fairly large number of experiments the value found for (cat min  $Cl + HCO_3$ ) in the dialysis solution was outside the range expected

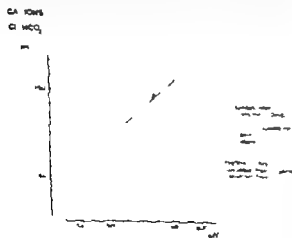


Fig 47 Relation between the values found for (cat min  $Cl + HCO_3$ ) in the dialysis solution and the values obtained for  $\Delta$  (cat min  $Cl + HCO_3$ ). See text.

from the known concentration of the other filtrable anions present in serum. Reasons were given why we assume that this unexplained discrepancy does not invalidate the

## *Results obtained with equilibrium dialysis*

Most experiments were done with serum; a smaller number with solutions of albumin and  $\gamma$  globulin. The few experiments in which plasma was dialysed will be presented together with the results obtained with serum of normal subjects.

### SERUM FROM NORMAL SUBJECTS

#### DATA CONCERNING SUBJECTS STUDIED — PRESENTATION OF RESULTS

23 dialysis experiments were done between November 1960 and February 1962. In 5 experiments plasma was dialysed; in 17 serum and in 1 experiment both plasma and serum. In 19 experiments the serum or plasma was obtained from one (fasting) subject but in 4 experiments pooled serum was studied, each sample containing serum from 3 (non fasting) subjects. The samples were dialysed directly after they were obtained. Since some subjects contributed more than once the total number of persons whose serum or plasma was studied is 19: one a female. All but 3 also underwent *in vivo* ultrafiltration and in most cases the sample used for dialysis was obtained immediately after the conclusion of the *in vivo* ultrafiltration experiment.

Nearly all serum samples were dialysed at three different pH values. The latter ranged from 6.33 to 7.96 but in all but 3 of the experiments the pH was within the range 6.60 to 7.80.

For the description of the method and the calculations involved the reader is referred to chapter V.

The results of the individual experiments (no correction for systematic errors) are given in table 47. For reasons of economy the small number of experiments in which pathological serum was dialysed have been listed at the end of table 47. They will be discussed separately. Table 47 needs some explanation.

The heading "distilled water" refers to the solution inside the control unit after dialysis, the heading "dialysis solution" to the composition of the solution outside the dialysis units at the end of the dialysis. Under the heading "protein solution" are indicated respectively: the number of dialysis units filled from the same serum (plasma) sample, whether the protein solution was plasma or serum, the mean pH value, the mean protein concentration and the mean values for NCE,  $\Delta$  Na,  $\Delta$  K and  $\Delta$  Ca + Mg for the number of units indicated (the maximum difference in protein concentration between the units filled from one sample was never more than 7 g per Kg H<sub>2</sub>O). For reasons discussed in chapter V only the results from units containing a solution with more than 50 g/Kg H<sub>2</sub>O protein have been included in table 47. As to the protein composition the values placed between brackets are the values found for plasma in the preceding *in vivo* ultra-

tration experiments. In most cases serum was dialysed but the relative percentages of albumin and the different globulins must have been practically the same as for plasma.

Finally in the columns concerning the data concerning the concentration ratios the heading distilled water refers to the distribution across the membrane of the control unit, the heading 'protein solution' to the distribution across the membrane of the serum or plasma containing units. In the latter case all ratios refer to a protein concentration of 70 g/Kg  $H_2O$  the reason for that will be discussed in the next section.

For Ca and Mg no separate results are given. In order to reduce the amount of analytical work only the sum concentration  $Ca + Mg$  was determined in all experiments but 4 and in these plasma was studied. The values obtained for  $\Delta Ca$  and  $\Delta Mg$  in these 4 experiments will be presented separately.

Concerning the interpretation of changes in  $Ca + Mg$  the following can be said. Both CARR (1955) and VAN OS & KOORMAN - VAN EUREN (1957) observed that albumin attracts  $Ca^{++}$  and  $Mg^{++}$  ions apparently with equal strength. Presumably therefore a given change in pH will reduce the same relative changes in albumin-bound  $Ca$  and in albumin-bound  $Mg$ . Although in serum the proteins bind a higher percentage of  $Ca$  than of  $Mg$  the difference is not large (see chapter VI) and in our dialysis experiments we can presumably interpret changes in the serum concentration of  $Ca + Mg$  as indicating equal (relative) changes in the concentration of each of the two cations.

#### SOME POINTS CONCERNING EXPERIMENTAL RESULTS AND THEIR PRESENTATION

Correlation between the values found for (cat min  $Cl + HCO_3$ ) in the dialysis solution and the ones calculated for  $\Delta$  (cat min  $Cl + HCO_3$ ) i.e. NCE.

In chapter V it was pointed out that in a fairly large number of experiments the value found for (cat min  $Cl + HCO_3$ ) in the dialysis solution was outside the range expected

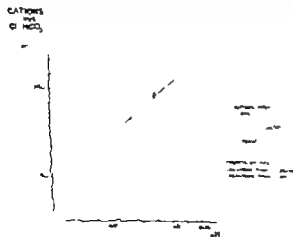


Fig 47 Relation between the values found for (cat min  $Cl + HCO_3$ ) in the dialysis solution and the values obtained for  $\Delta$  (cat min  $Cl + HCO_3$ ) see text.

from the known concentration of the other filtrable anions present in serum. Reasons were given why we assume that this unexplained discrepancy does not invalidate the

determination of NCE. We are now in a position to test this assumption in the following manner. In fig. 47 all values obtained for  $\Delta$  (cat min Cl +  $\text{HCO}_3$ ) with normal serum or plasma are plotted against pH using different symbols to indicate whether the value actually found for (cat min Cl +  $\text{HCO}_3$ ) in the dialysis solution was higher than expected, as expected or lower than expected. It will be seen that the three groups of symbols intermingle completely. Exclusion of all experiments in which the value of (cat min Cl +  $\text{HCO}_3$ ) in dialysis solution was outside the probable range - i.e. differed by more than 2 SD from the expected value - has virtually no influence on slope and position of the regression line calculated for  $\Delta$  (cat min Cl +  $\text{HCO}_3$ ) on pH.

The regression lines were calculated by the method of least squares and they only serve as a means for comparing the two groups of experimental points. It is not suggested that they indicate the true relationship between pH and NCE, which will be discussed later.

### Necessity of expressing concentration ratios at a uniform protein concentration

The concentration ratio of a particular ion depends on the concentration of its 'free fraction' and on protein net charge. The first has been fairly constant in our experiments, but the second differed considerably as a result of differences both in total protein concentration and in pH (for the normal subjects protein composition was sufficiently similar to be considered constant). If we want to study the effect of changes in pH on the concentration ratios, the effect of a change in protein concentration has to be eliminated by calculating the ratios at a uniform protein concentration. For the latter we have chosen the value of 70 g/Kg  $\text{H}_2\text{O}$ . At this protein concentration the concentration ratios will be comparable with the values published previously for the concentration ratios at the boundary serum - interstitial fluid.

The ratio to be expected at a protein concentration of 70 g/Kg  $\text{H}_2\text{O}$  was obtained in the following manner (Na is taken for an example) from  $[\text{Na}]_i$ ,  $[\text{Na}]_o$  and the actual protein concentration.  $\Delta$  Na is calculated and expressed per 70 g protein. By adding this value to the concentration found in the dialysis solution we can calculate both the Na concentration inside and the concentration ratio to be expected at a protein content of 70 g/Kg  $\text{H}_2\text{O}$ . For the anions the concentration inside decreases with increasing protein concentration, but otherwise the procedure is the same. Its justification is to be found in chapter V where it was shown that over the range 15 to 70 g/Kg  $\text{H}_2\text{O}$  protein the  $\Delta$  values are linearly related to the protein concentration inside the unit with the possible exception of  $\Delta$  Cl and  $\Delta$  (cat min Cl +  $\text{HCO}_3$ ). But for the protein range, with which we are here concerned, i.e. 52 to 74 g/Kg  $\text{H}_2\text{O}$  the linearity of the relation seems well established even for  $\Delta$  Cl.

We can therefore write

$$[\text{Cat}]_i = [\text{Cat}]_o + 0.01 \Delta \text{ Cat } [\text{Pr}]_i$$

where  $\Delta$  Cat stands for  $\Delta$  Cat expressed per 100 g protein and  $[\text{Pr}]_i$  for the actual protein concentration inside the dialysis unit. The concentration ratio is then

$$R_{\text{cat}} = \frac{[\text{Cat}]_i}{[\text{Cat}]_o} = \frac{[\text{Cat}]_o}{[\text{Cat}]_o + 0.01 \Delta \text{ Cat } [\text{Pr}]_i}$$

Similarly we find

$$R_{\text{an}} = \frac{[\text{An}]_i}{[\text{An}]_o} = \frac{[\text{An}]_o - 0.01 \Delta \text{ An } [\text{Pr}]_i}{[\text{An}]_o}$$

Album.  
1000La  
1000

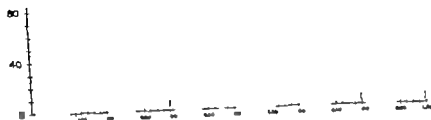
Ca

Vro  
1000vdP  
1000

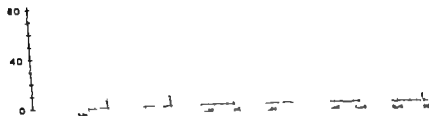
vdB

TEIN  
g H<sub>2</sub>O

Na



Cl



CONCENTRATION RATIO

ROTEIN  
g H<sub>2</sub>O

Ca + Mg



CONCENTRATION RATIO

Fig. 43. Relation between protein concentration and concentration ratios of Na, Cl and Ca + Mg. The diagram has no statistical meaning but simply connects the average concentration ratio found at the highest protein concentration with the point representing concentration ratio 100 at protein concentration zero.

As long as the concentration of  $[\text{Cat}]_0$  is large in comparison to  $\Delta \text{Cat}$  (as in the case of Na and K) the relation between concentration ratio and protein concentration is practically linear for protein concentrations up to 100 g/Kg  $\text{H}_2\text{O}$ . But if  $\Delta \text{Cat}$  is of the same magnitude as  $[\text{Cat}]_0$  - as in the case of Ca and Mg - the relation is curvilinear.

In chapter I a similar relation was deduced for the Donnan ratio and fig. 48 shows for Na, Cl and Ca + Mg the relation experimentally found in the experiments in which various dilutions of the serum were dialysed in one container. It can be seen that for Na and Cl the relationship is indeed rectilinear within the error of determination, whereas for Ca + Mg the curvilinearity is evident. K and  $\text{HCO}_3$  are not shown because of the much larger spread in their case - but the position of the points was found compatible with rectilinearity.

From fig. 48 the necessity of expressing concentration ratios at uniform protein concentration should be evident, and since in the experiments represented in table 47 the actual protein concentration was always higher than 50 g/Kg  $\text{H}_2\text{O}$  the increase in inaccuracy resulting from the extrapolation to protein concentration 70 g/Kg  $\text{H}_2\text{O}$  was but small.

#### CORRECTION FOR SYSTEMATIC ERRORS

Two of the errors mentioned when presenting the results of the *in vitro* ultrafiltration experiments may be expected to affect also the results obtained by means of dialysis.

(a) *Flame photometer error in determination of Na and K.* At a protein concentration of about 60 g/Kg  $\text{H}_2\text{O}$  this should lead to underestimating both Na and K concentration in serum by 0.1 mEq per Kg  $\text{H}_2\text{O}$ . As a consequence  $\Delta \text{Na}$  and  $\Delta \text{K}$ , when expressed per 100 g protein, are each underestimated by 0.15 mEq, and NCE by 0.3 mEq. This error is presumably independent of the pH of the solution analysed.

(b) *Error due to neglect of the presence of carbonate and of carbamino-protein.* Since NCE etc. are calculated from the differences between the concentrations inside and outside the dialysis units, neglect of the presence of carbonate should cause no error. This leaves the error due to neglect of carbamino-protein. For reasons given in chapter III we have assumed that at physiological pH and  $\text{Pco}_2$  sufficient carbamino-protein is present to make us overestimate  $\text{HCO}_3$  concentration by 0.3 mEq at a protein concentration of 100 g/Kg  $\text{H}_2\text{O}$ . If we accept the existence of this amount of carbamino-protein at pH 7.40, we must expect that below pH 7.00 no carbamino-protein exists, but that at pH 8.00 the concentration will at least be twice that present at pH 7.40 (see experiments of Grassia *et al* 1953).

We conclude that both  $\Delta \text{Na}$  and  $\Delta \text{K}$  are on the average 0.15 mEq too low when expressed per 100 g protein, and that  $\Delta \text{HCO}_3$  will be correct below pH 7.00, but is overestimated by about 0.3 mEq at pH 7.40 and by about 0.6 mEq at pH 8.00. As a consequence NCE should be underestimated by 0.3 mEq below pH 7.00, by 0.6 mEq at pH 7.40 and by 0.9 mEq at pH 8.00, all values expressed per 100 g protein.

In the following sections only the average values will be corrected for these errors.

#### RESULTS

These may be divided into those concerning NCE and  $\Delta \text{cation}$  and those concerning the concentration ratios. Henceforward the terms NCE and  $\Delta \text{cation}$  refer to quantities

expressed per 100 g protein, unless otherwise stated. Similarly the term concentration ratio will indicate the ratio at a protein concentration of 70 g/Kg H<sub>2</sub>O

### NCE and $\Delta$ cation

By expressing all quantities per 100 g protein differences due to variation in actual protein concentration are eliminated and for normal sera the differences in protein composition may be considered negligible. But the effect of pH remains and must be taken into account when comparing the values found for NCE or  $\Delta$  cation.

### NCE

Fig. 49 (left-hand side) graphically presents the values found for NCE in relation to the pH of the serum or plasma (the three experiments in table 47 in which the latter was not determined are not included).

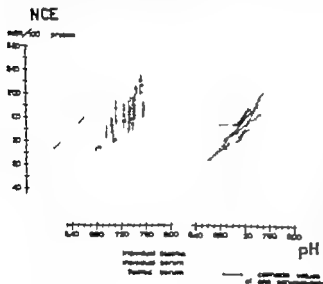


Fig. 49 Normal serum and plasma. Correlation between pH and NCE (values not corrected for systematic errors). On the left side all experiments are represented, on the right side only those in which the same serum sample was dialysed at different pH values. The broken line represents the values calculated from the equation given by V. SLYKE *et al.* in 1928. For further explanation see text.

For each point in the graph the SE was calculated from the SD found for single determinations of NCE (table 20 in chapter V) and from the number of units averaged for the calculation of that particular point. For NCE the range covered by  $\pm 2$  SE is indicated by the vertical lines projecting upwards and downwards from the symbols. For pH the range  $\pm 2$  SE is indicated by the diameter of the symbol. The broken line represents the relationship between NCE and pH according to the 1928 publication of VAN SLYKE *et al.* (equation D in chapter II).

In fig. 49 (right-hand side) only the values obtained for sera which were dialysed at more than one pH value are given. The values found for one serum during one dialysis experiment are connected.

The impression gained from fig. 49 is that the values found for NCE are considerably below the values expected from Van Slyke's experiments, but that the change per unit pH is about the same.



As long as the concentration of  $[\text{Cat}]_0$  is large in comparison to  $\Delta \text{Cat}$  (as in the case of Na and K) the relation between concentration ratio and protein concentration is practically linear for protein concentrations up to 100 g/Kg  $\text{H}_2\text{O}$ . But if  $\Delta \text{Cat}$  is of the same magnitude as  $[\text{Cat}]_0$  – as in the case of Ca and Mg – the relation is curvilinear.

In chapter I a similar relation was deduced for the Donnan ratio and fig. 48 shows for Na + Cl and Ca + Mg the relation experimentally found in the experiments in which various dilutions of the serum were dialysed in one container. It can be seen that for Na and Cl the relationship is indeed rectilinear within the error of determination whereas for Ca + Mg the curvilinearity is evident. K and  $\text{HCO}_3$  are not shown because of the much larger spread in their case – but the position of the points was found compatible with rectilinearity.

From fig. 48 the necessity of expressing concentration ratios at uniform protein concentration should be evident and since in the experiments represented in table 47 the actual protein concentration was always higher than 50 g/Kg  $\text{H}_2\text{O}$  the increase in inaccuracy resulting from the extrapolation to protein concentration 70 g/Kg  $\text{H}_2\text{O}$  was but small.

### CORRECTION FOR SYSTEMATIC ERRORS

Two of the errors mentioned when presenting the results of the *in vivo* ultrafiltration experiments may be expected to affect also the results obtained by means of dialysis.

(a) *Flame photometer error in determination of Na and K* At a protein concentration of about 60 g/Kg  $\text{H}_2\text{O}$  this should lead to underestimating both Na and K concentration in serum by 0.1 mEq per Kg  $\text{H}_2\text{O}$ . As a consequence  $\Delta \text{Na}$  and  $\Delta \text{K}$ , when expressed per 100 g protein, are each underestimated by 0.15 mEq, and NCE by 0.3 mEq. This error is presumably independent of the pH of the solution analysed.

(b) *Error due to neglect of the presence of carbonate and of carbamino-protein* Since NCE etc. are calculated from the differences between the concentrations inside and outside the dialysis units, neglect of the presence of carbonate should cause no error. This leaves the error due to neglect of carbamino-protein. For reasons given in chapter III we have assumed that at physiological pH and  $\text{Pco}_2$  sufficient carbamino-protein is present to make us overestimate  $\text{HCO}_3$  concentration by 0.3 mEq at a protein concentration of 100 g/Kg  $\text{H}_2\text{O}$ . If we accept the existence of this amount of carbamino-protein at pH 7.40 we must expect that below pH 7.00 no carbamino-protein exists, but that at pH 8.00 the concentration will at least be twice that present at pH 7.40 (see experiments of GIUSTINA *et al.* 1953).

We conclude that both  $\Delta \text{Na}$  and  $\Delta \text{K}$  are on the average 0.15 mEq too low when expressed per 100 g protein, and that  $\Delta \text{HCO}_3$  will be correct below pH 7.00, but is overestimated by about 0.3 mEq at pH 7.40 and by about 0.6 mEq at pH 8.00. As a consequence NCE should be underestimated by 0.3 mEq below pH 7.00, by 0.6 mEq at pH 7.40 and by 0.9 mEq at pH 8.00, all values expressed per 100 g protein.

In the following sections only the average values will be corrected for these errors.

### RESULTS

These may be divided into those concerning NCE and  $\Delta$  cation and those concerning the concentration ratios. Henceforward the terms NCE and  $\Delta$  cation refer to quantities

Accordingly at pH 7.33 NCE would be calculated at 16.3 mEq/100 g protein. The agreement with the corresponding (non-corrected) mean values calculated from the serum samples in the pH range 7.10-7.50 (table 48) is again rather satisfactory.

A correction for the effect of the systematic errors can be made in the following manner. The error in flame photometry is independent of pH and should therefore not affect the slope of the regression line, but only the intercept with the horizontal axis *i.e.* the apparent IEP. But the error due to carbamino-protein was assumed to be zero below pH 7.00 and 0.6 mEq/100 g protein at pH 8.00. A correction for this error must therefore increase the slope of the line in the pH range 7.00 to 8.00. Finally although in the experiments used for calculating the average regression line the pH ranged from 6.33 to 7.96, all but three points fall within the range 6.60 to 7.70. Thus we found the best estimate of the relationship between pH and NCE for the pH range 6.60-7.00

$$\text{NCE (mEq/100 g protein)} = 10.0 (\text{pH} - 5.66),$$

for the pH range 7.00-7.70

$$\text{NCE (mEq/100 g protein)} = 10.7 (\text{pH} - 5.74).$$

These equations should be valid for serum with normal protein composition and normal electrolyte concentrations.

### $\Delta$ cation

In fig. 50 we have presented the data for  $\Delta$  Na,  $\Delta$  K and  $\Delta$  Ca + Mg in the same manner as for NCE. On the left-hand side all values ( $\pm 2$  SE of the mean) for which the pH in serum or plasma was determined are presented, on the right hand side only the results for serum samples dialysed at more than one pH value. For  $\Delta$  K and  $\Delta$  Ca + Mg some further comment is necessary.

In their case the concentration of 'free' ion in the individual experiments differed by as much as 15% from the mean concentration calculated for all experiments, whereas for Na the difference was always less than 3%. Since large changes in the concentration of 'free' ion more affect the correlation between pH and  $\Delta$  cation, the right-hand side of fig. 50 contains in the case of  $\Delta$  Ca + Mg only the experiments in which the concentration of Ca + Mg in the dialysis solution was within the range 4.00 to 4.20 mEq/Kg H<sub>2</sub>O (mean concentration 4.06). This spread is comparable to that found for the Na concentration of the dialysis solution.

The K concentration in the stock dialysis solution was changed after 14.5.56 from 3.90 mEq/L to 4.75 mEq/L in an attempt to make the influence of the absolute error smaller. But for K the percentage error remained considerably larger than for Na and, together with the fact that the flame photometer error is also considerably larger than in the case of Na, this greatly limits the accuracy of the results obtained for K. Although fig. 50 (right-hand side) in the case of K only presents the experiments in which the concentration in the dialysis solution was between 4.69 and 5.06 mEq/Kg H<sub>2</sub>O no definite pattern emerges and we shall therefore not attempt to correlate  $\Delta$  K with pH.

Table 50 gives the ranges of the values obtained for  $\Delta$  Na,  $\Delta$  K and  $\Delta$  Ca + Mg in the same 14 experiments with serum from which the mean NCE value for the pH range 7.10-7.50 was calculated. For each cation the average concentration in the dialysis solution is also given. Comparing these results with those obtained for protein-bound Na and Ca + Mg by means of *in vivo* ultrafiltration (chapter VI), we find that the values found with dialysis are lower although the concentration of 'free' cation was about the

*Average value of NCE at pH of normal venous blood* In the *in vivo* ultrafiltration experiments endcompression pH ranged from 7.27 to 7.35 in the normal persons. The number of dialysis experiments in which the pH inside the serum or plasma containing unit was within this range is only 5 but if we assume a linear relationship between pH and NCE for the pH range 7.10–7.50 – and this assumption is not unreasonable as fig. 49 shows – 14 observations with serum and 3 with plasma become available for comparison with the results obtained by means of *in vivo* ultrafiltration.

The experiments have been selected as follows. When for one serum sample two experimental points fall within the pH range indicated the value with the pH nearest to 7.30 was chosen. And when serum of the same individual was studied more than once only the result of the first experiment was taken. The pooled sera have all been included, although they contained serum of at least one of the subjects of whom also an individual serum is included in the series. The experiments thus include serum or plasma from 15 different subjects.

In table 48 the experiments are specified according to the solution studied. The sera show the lowest average value and plasma the highest. The small number of experiments, the difference in mean pH value and the fact that the samples were not obtained from the same subjects prevent the difference between serum and plasma from being statistically significant. It is however in the direction expected from the estimated contribution of heparin to NCE (chapter V) and we have therefore considered the serum values to be the more accurate of the two.

Correction for systematic errors brings the mean NCE value of the serum experiments from 16.4 to 17.0 mEq/100 g protein at a mean pH value of 7.33. This value is in close agreement with the average value calculated from the *in vivo* ultrafiltration experiments after correction for systematic errors (chapter VI). In chapter VIII the results of both methods will be compared in more detail.

If all observations in which the pH of the protein solution was determined are averaged we find for a mean pH value of 7.17 a mean NCE value of 14.7 (for a total of 45 experiments in which the pH ranged from 6.33 to 7.96). Assuming NCE to increase 10 mEq per 100 g protein per unit pH (see page 39) we calculate a (non-corrected) NCE value of 16.3 at pH 7.33. The difference with the non-corrected value obtained from the 14 experiments in the pH range 7.10–7.50 is negligible and for practical purposes the assumption of a rectilinear relationship between pH and NCE over the pH range studied appears to be justified.

Finally it should be mentioned that for the one subject (vLe) from whom serum as well as plasma was studied on several occasions the values found for NCE were equal within the error of determination. Obviously the error caused by the addition of heparin will only become evident if a larger number of experiments is compared.

*Correlation between pH and NCE* We have calculated the regression line for NCE on pH for each of the 15 serum samples which were dialysed at more than one pH value. They include 4 samples of pooled serum. The individual equations are presented in table 49 together with the average equation. The slopes of the individual regression lines vary considerably owing to the small number of observations per serum and to the difference in pH range covered. But the average equation should be a reasonable approximation of the correlation in our experiments. From table 49 we find

$$\text{NCE (mEq/100 g protein)} = 10.0 (\text{pH} - 5.70)$$

Accordingly at pH 7.33 NCE would be calculated at 16.3 mEq/100 g protein. The agreement with the corresponding (non-corrected) mean values calculated from the serum samples in the pH range 7.10-7.50 (table 48) is again rather satisfactory.

A correction for the effect of the systematic errors can be made in the following manner. The error in flame photometry is independent of pH and should therefore not affect the slope of the regression line, but only the intercept with the horizontal axis *i.e.* the apparent IEP. But the error due to carbamino-protein was assumed to be zero below pH 7.00 and 0.6 mEq/100 g protein at pH 8.00. A correction for this error must therefore increase the slope of the line in the pH range 7.00 to 8.00. Finally although in the experiments used for calculating the average regression line the pH ranged from 6.33 to 7.96 all but three points fall within the range 6.60 to 7.70. Thus we found the best estimate of the relationship between pH and NCE for the pH range 6.60-7.00

$$\text{NCE (mEq/100 g protein)} = 10.0 (\text{pH} - 5.66),$$

for the pH range 7.00-7.70

$$\text{NCE (mEq/100 g protein)} = 10.7 (\text{pH} - 5.74).$$

These equations should be valid for serum with normal protein composition and normal electrolyte concentrations.

#### $\Delta$ cation

In fig. 50 we have presented the data for  $\Delta$  Na,  $\Delta$  K and  $\Delta$  Ca + Mg in the same manner as for NCE. On the left hand side all values ( $\pm 2$  SE of the mean) for which the pH in serum or plasma was determined are presented. On the right-hand side only the results for serum samples dialysed at more than one pH value. For  $\Delta$  K and  $\Delta$  Ca + Mg some further comment is necessary.

In their case the concentration of free ion in the individual experiments differed by as much as 15% from the mean concentration calculated for 8 experiments, whereas for Na the difference was always less than 3%. Since large changes in the concentration of free ion must affect the correlation between pH and  $\Delta$  cation, the right-hand side of fig. 50 contains in the case of  $\Delta$  Ca + Mg only the experiments in which the concentration of Ca + Mg in the dialysis solution was within the range 4.00 to 4.20 mEq/Kg H<sub>2</sub>O (mean concentration 4.08). This spread is comparable to that found for the Na concentration of the dialysis solution.

The K concentration in the stock dialysis solution was changed after 14-5-'61 from 3.50 mEq/L to 4.75 mEq/L in an attempt to make the influence of the absolute error smaller. But for K the percentage error remained considerably larger than for Na and, together with the fact that the flame photometer error is also considerably larger than in the case of Na, this greatly limits the accuracy of the results obtained for K. Although fig. 50 (right-hand side) in the case of K only presents the experiments in which the concentration in the dialysis solution was between 4.69 and 5.06 mEq/Kg H<sub>2</sub>O no definite pattern emerges and we shall therefore not attempt to correlate  $\Delta$  K with pH.

Table 50 gives the averages of the values obtained for  $\Delta$  Na,  $\Delta$  K and  $\Delta$  Ca + Mg in the same 14 experiments with serum from which the mean NCE value for the pH range 7.10-7.50 was calculated. For each cation the average concentration in the dialysis solution is also given. Comparing these results with those obtained for protein-bound Na and Ca + Mg by means of *in vivo* ultrafiltration (chapter VI), we find that the values found with dialysis are lower although the concentration of free cation was about the

*Average value of NCE at pH of normal venous blood* In the *in vivo* ultrafiltration experiments endcompression pH ranged from 7.27 to 7.35 in the normal persons. The number of dialysis experiments in which the pH inside the serum or plasma containing unit was within this range is only 5 but if we assume a linear relationship between pH and NCE for the pH range 7.10–7.50 – and this assumption is not unreasonable as fig. 49 shows – 14 observations with serum and 3 with plasma become available for comparison with the results obtained by means of *in vivo* ultrafiltration.

The experiments have been selected as follows. When for one serum sample two experimental points fall within the pH range indicated the value with the pH nearest to 7.30 was chosen. And when serum of the same individual was studied more than once only the result of the first experiment was taken. The pooled sera have all been included, although they contained serum of at least one of the subjects of whom also an individual serum is included in the series. The experiments thus include serum or plasma from 15 different subjects.

In table 48 the experiments are specified according to the solution studied. The sera show the lowest average value and plasma the highest. The small number of experiments, the difference in mean pH value and the fact that the samples were not obtained from the same subjects prevent the difference between serum and plasma from being statistically significant. It is, however, in the direction expected from the estimated contribution of heparin to NCE (chapter V) and we have therefore considered the serum values to be the more accurate of the two.

Correction for systematic errors brings the mean NCE value of the serum experiments from 16.4 to 17.0 mEq/100 g protein at a mean pH value of 7.33. This value is in close agreement with the average value calculated from the *in vivo* ultrafiltration experiments after correction for systematic errors (chapter VI). In chapter VIII the results of both methods will be compared in more detail.

If all observations in which the pH of the protein solution was determined are averaged we find for a mean pH value of 7.17 a mean NCE value of 14.7 (for a total of 45 experiments in which the pH ranged from 6.33 to 7.96). Assuming NCE to increase 10 mEq per 100 g protein per unit pH (see page 39) we calculate a (non-corrected) NCE value of 16.3 at pH 7.33. The difference with the non-corrected value obtained from the 14 experiments in the pH range 7.10–7.50 is negligible and for practical purposes the assumption of a rectilinear relationship between pH and NCE over the pH range studied appears to be justified.

Finally it should be mentioned that for the one subject (vLe) from whom serum as well as plasma was studied on several occasions the values found for NCE were equal within the error of determination. Obviously the error caused by the addition of heparin will only become evident if a larger number of experiments is compared.

*Correlation between pH and NCE.* We have calculated the regression line for NCE on pH for each of the 15 serum samples which were dialysed at more than one pH value. They include 4 samples of pooled serum. The individual equations are presented in table 49 together with the average equation. The slopes of the individual regression lines vary considerably owing to the small number of observations per serum and to the difference in pH range covered. But the average equation should be a reasonable approximation of the correlation in our experiments. From table 49 we find

$$\text{NCE (mEq/100 g protein)} = 10.0 (\text{pH} - 5.70)$$

Accordingly at pH 7.33 NCE would be calculated at 16.3 mEq/100 g protein. The agreement with the corresponding (non-corrected) mean values calculated from the serum samples in the pH range 7.10-7.50 (table 48) is again rather satisfactory.

A correction for the effect of the systematic errors can be made in the following manner. The error in flame photometry is independent of pH and should therefore not affect the slope of the regression line, but only the intercept with the horizontal axis *i.e.* the apparent IEP. But the error due to carbamino-protein was assumed to be zero below pH 7.00 and 0.6 mEq/100 g protein at pH 8.00. A correction for this error must therefore increase the slope of the line in the pH range 7.00 to 8.00. Finally, although in the experiments used for calculating the average regression line the pH ranged from 6.33 to 7.96, all but three points fall within the range 6.60 to 7.70. Thus we found the best estimate of the relationship between pH and NCE for the pH range 6.60-7.00

$$\text{NCE (mEq/100 g protein)} = 10.0 (\text{pH} - 5.66),$$

for the pH range 7.00-7.70

$$\text{NCE (mEq/100 g protein)} = 10.7 (\text{pH} - 5.74).$$

These equations should be valid for serum with normal protein composition and normal electrolyte concentrations.

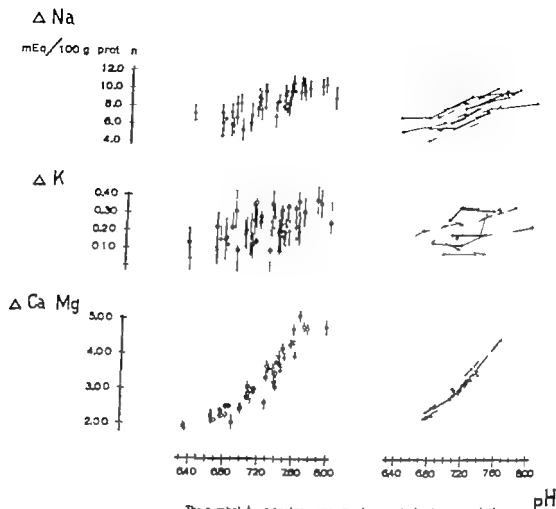
#### $\Delta$ cation

In fig. 50 we have presented the data for  $\Delta$  Na,  $\Delta$  K and  $\Delta$  Ca + Mg in the same manner as for NCE. On the left-hand side all values ( $\pm 2$  SE of the mean) for which the pH in serum or plasma was determined are presented, on the right-hand side only the results for serum samples dialysed at more than one pH value. For  $\Delta$  K and  $\Delta$  Ca + Mg some further comment is necessary.

In the case the concentration of 'free' ion in the individual experiments differed by as much as 15% from the mean concentration calculated for all experiments, whereas for Na the difference was always less than 3%. Since large changes in the concentration of 'free' ion must affect the correlation between pH and  $\Delta$  cation, the right-hand side of fig. 50 consists in the case of  $\Delta$  Ca + Mg only the experiments in which the concentration of Ca + Mg in the dialysis solution was within the range 4.00 to 4.20 mEq/Kg H<sub>2</sub>O (mean concentration 4.08). This spread is comparable with that found for the Na concentration of the dialysis solution.

The K concentration in the stock dialysis solution was changed after 14-5-61 from 3.50 mEq/L to 4.5 mEq/L as an attempt to make the influence of the absolute error smaller. But for K the percentage error remained considerably larger than for Na and, together with the fact that the flame photometric error is also considerably larger than in the case of Na, this greatly limits the accuracy of the results obtained for K. Although fig. 50 (right-hand side) in the case of K only presents the experiments in which the concentration in the dialysis solution was between 4.69 and 5.06 mEq/Kg H<sub>2</sub>O no definite pattern emerges and shall therefore not attempt to correlate  $\Delta$  K with pH.

Table 50 gives the averages of the values obtained for  $\Delta$  Na,  $\Delta$  K and  $\Delta$  Ca + Mg in the same 14 experiment with serum from which the mean NCE value for the pH range 7.10-7.50 was calculated. For each cation the average concentration in the dialysis solution is also given. Comparing these results with those obtained for protein-bound Na and Ca + Mg by means of *in situ* ultrafiltration (chapter VI), we find that the values found with dialysis are lower although the concentration of 'free' cation was about the



The symbol  $\phi$  indicates experiments in which the concentration of the dialysis solution was  
for K 4.00 — 5.00 mEq/Kg  $H_2O$   
and for  $Ca + Mg$  4.0 — 4.19 mEq/Kg  $H_2O$

In both cases only these experiments were used for the construction of the graphs on the righthand side.

Fig. 50 — Normal serum and plasma. Correlation between pH and respectively  $\Delta Na$ ,  $\Delta Ca + Mg$  (does not corrected for systematic errors). See subscript fig. 49 and text for explanation.

same. A further discussion will follow in chapter VIII but it can already be said here that the discrepancy must be due to the effect of the Donnan equilibrium.

*Correlation between pH and  $\Delta$  cation* Fig. 50 demonstrates that whereas for  $\Delta Na$  the correlation is roughly rectilinear it is clearly curvilinear for  $\Delta Ca + Mg$ .

For  $\Delta Na$  we have calculated the following average regression line from the same 15 experiments as for NCE

$$\Delta Na \text{ (mEq/100 g protein)} = 4.00 (\text{pH} - 5.08)$$

For the individual regression lines the correlation coefficient ranged from 2.34 to 5.54. If we correct for the error in flame photometry the apparent IEP becomes 5.10.

For  $\Delta Ca + Mg$  we have calculated the line of best fit in the following manner. First

the data from experiments in which the 'free'  $\text{Ca} + \text{Mg}$  concentration was between 4.00 and 4.26 mEq/Kg  $\text{H}_2\text{O}$  were grouped according to pH, and for each range a mean value was calculated (only experiments with serum were included). Then the same was done for all experiments. Table 51 shows that the mean concentration of  $\text{Ca} + \text{Mg}$  in the dialysis solution was the same for both groups of experiments and that practically the same mean values were found both for  $\Delta \text{Ca} + \text{Mg}$  and for pH in all pH ranges (fig. 51). Whereas between pH 6.60 and 6.80  $\Delta \text{Ca} + \text{Mg}$  increases by about 0.1 mEq

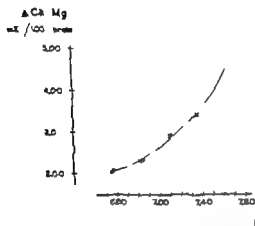


Fig. 51. Normal serum. Correlation between mean values of pH and of  $\Delta \text{Ca} + \text{Mg}$  for different pH ranges. The crosses refer to the values given in table 51 column I, and the open circles to those given in table 51 column II.

per 0.1 pH unit, the increase is 0.2 mEq over the pH range 7.00–7.20 and something like 0.4 mEq over the range 7.40–7.60. However within the pH range 7.00–7.50 the relationship can be described approximately by the equation

$$\Delta \text{Ca} + \text{Mg} (\text{mEq}/100 \text{ g protein}) = 2.50 (\text{pH} - 5.94).$$

Finally it must be stressed that in order to obtain the correlation between protein-bound cation and pH the observed correlation between  $\Delta$  cation and pH has yet to be corrected for the effect of the Donnan equilibrium.

### Concentration ratios

Table 47 gives both the ratios obtained with serum or plasma and those obtained with distilled water. The latter should be 1.000 within the error of the determination and thus serve as a 'blank' for the ratios found in the case of serum. Fig. 52 shows for each of the ions studied the relationship between concentration ratio and pH.

All experiments have been included with the exception of those in which the pH of the serum or plasma was not determined. The points refer to 'distilled water' the crosses to serum or plasma. Crosses representing the same serum sample are connected.

It is obvious that the values obtained for  $R_{\text{K}}$  and for  $R_{\text{MgCO}_3}$  are subject to a large accidental error but for  $R_{\text{Na}}$ ,  $R_{\text{Cl}}$  and  $R_{\text{Ca} + \text{Mg}}$  the spread is relatively small in comparison to the influence of pH. In the case of these ions even the individual serum samples show roughly linear correlation between concentration ratio and pH. We have therefore



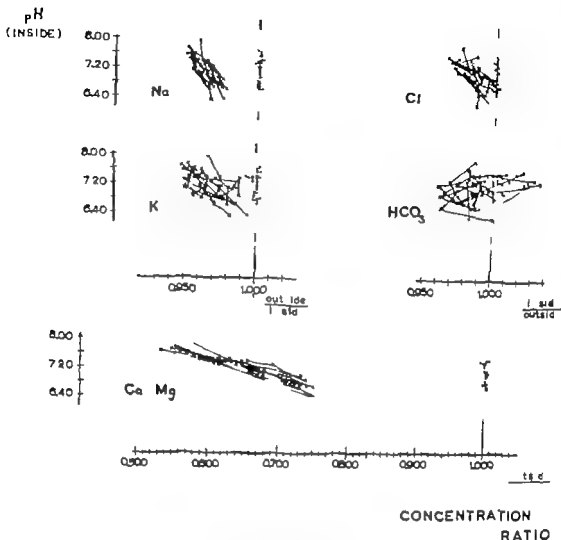


Fig. 52 Normal serum and plasma. Correlation between pH and the concentration ratios for the equilibrium dialysis solution serum (crosses) and for the equilibrium dialysis solution distilled water (dots). V has not corrected for systematic errors.

divided our observations on serum in two groups according to pH value. For the pH ranges 6.60–7.20 respectively 7.20–7.75 mean values were calculated for each of the concentration ratios and for the pH by simply averaging the values within one group. The results are presented in table 52. Since these data will later be used for the calculation of the amount of ion that is bound in a complex-type manner (chapter VIII), we have estimated the SE of the mean for the concentration ratio of each ion.

For the equilibrium dialysis solution-serum we proceeded as follows. On the assumption that within the pH range 6.60 to 7.75 a rectilinear correlation exists between pH and the mean value of the concentration ratio of the different ions we have calculated for  $R_{Na}$ ,  $R_{K}$  etc. the mean value to be expected at the pH value of each individual experiment from the mean values given in table 52 for pH 6.91 and 7.46 respectively. We then calculated for every individual value of  $R_{Na}$ ,  $R_{K}$  etc. the deviation from the mean at the particular pH value. Knowing these we could calculate for each of the concentration ratios the SD of an individual value and the SE of the mean for the pH ranges 6.60–7.20 and 7.20–7.75. This manner of calculating the spread is rather crude and in the case of  $R_{Ca}$  the assumption of a rectilinear correlation with pH is only correct for the

smaller pH range 7.00 to 7.30. Nevertheless the values estimated for the SE of the mean should be reasonably accurate, since we found nearly the same values when we calculated for  $R_{Na}$ ,  $R_{Cl}$  etc the SE of the mean to be expected from accidental errors (for which calculation we used the data given in tables 22, 30 and 31 in chapter V).

The ratios for the equilibrium dialysis solution "distilled water" are not affected by pH and in this case the SE of the mean could be calculated for  $R_{Na}$ ,  $R_{Cl}$  etc in the usual manner.

Table 52 confirms the impression gained from fig. 52. For the equilibrium dialysis solution-distilled water the mean values of the ratios are well within probable range of 1.000. For the equilibrium dialysis solution-serum we find in the case of  $R_{Na}$ ,  $R_{Cl}$  and  $R_{Ca+Mg}$  an apparently significant difference between the mean values calculated for the two different pH ranges, but in the case of  $R_{HCO_3}$  the spread is very large. We can only conclude that on the average  $R_{HCO_3}$  appears to be of the same order as  $R_{Cl}$ . For  $R_K$  the situation is complicated by the fact that part of the experiments were performed at a "free" K concentration of about 3.50 mEq/Kg  $H_2O$  the remainder at a concentration of about 4.75 mEq/Kg  $H_2O$ . But this difficulty is a minor one compared with the problem of the systematic errors.

Table 53 shows the effect of correcting the mean values of  $R_{Na}$  and of  $R_K$  for the flame photometer error. In the case of  $R_K$  the magnitude of the correction is less than 0.1%, but for  $R_K$  it amounts to about 2% of the mean value. The fact that the magnitude of the flame photometer error is less well substantiated for K than for Na further increases the inaccuracy of the values obtained in the case of K. As to the systematic error due to neglect of the presence of carbamino-protein, it must be stated again that the magnitude of the correction is very much an estimate. But the general effect of such a correction would be to decrease the mean concentration ratio of  $HCO_3^-$  at pH 7.44 to about 0.975 and to leave the concentration ratio at pH 6.91 unaffected.

On the assumption that for  $R_{Na}$ ,  $R_{Cl}$  and  $R_{Ca+Mg}$  a rectilinear relationship exists with pH over the pH range 6.90 to 7.40 we could make an estimate of the correlation with the aid of table 52. Thus we found

$$R_{Na} = 1.000 - 0.018 (\text{pH} - 4.98)$$

$$R_{Ca+Mg} = 1.000 - 0.185 (\text{pH} - 5.30)$$

$$R_{Cl} = 1.000 - 0.029 (\text{pH} - 6.60)$$

The most interesting finding is that whereas  $R_{Na}$  and  $R_{Ca+Mg}$  become 1.000 at a pH value near 5.00, the corresponding pH value is 6.60 in the case of  $R_{Cl}$ . But a detailed discussion of the implications of the results obtained for the concentration ratios must wait till chapter VIII.

Data concerning  $\Delta Ca$  and  $\Delta Ca + Mg$ , and  $R_{Ca}$  and  $R_{Mg}$

In four experiments with plasma both Ca and Ca + Mg were determined. The results are summarised in table 54. When the effect of pH is taken into account both for Ca and for Mg about the same percentage binding is found as calculated from the *in vivo* dialysation experiments (table 43 in chapter VI). Since relatively more Ca than Mg is bound,  $R_{Ca}$  is somewhat lower than  $R_{Mg}$ .

## SERUM FROM PATIENTS

Only four patients were studied but the results obtained merit some attention.

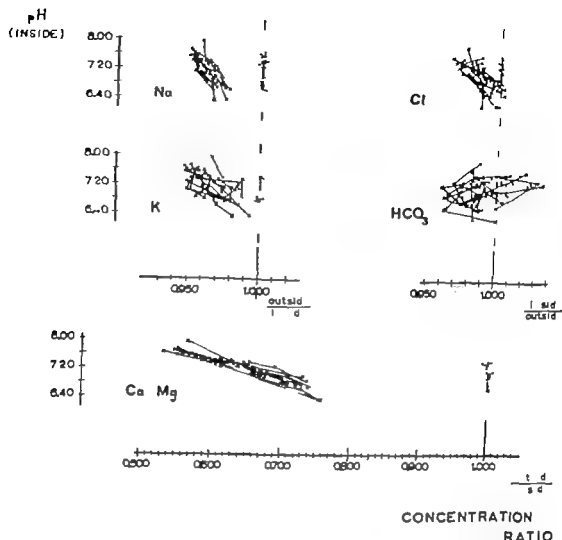


Fig. 52 Normal serum and plasma. Correlation between pH and the concentration ratios for the equilibrium dialysis solution - serum (crosses) and for the equilibrium dialysis solution - distilled water (points). Values not corrected for systematic errors.

divided our observations on *serum* in two groups according to pH value. For the pH ranges 6.60-7.20 respectively 7.20-7.75 mean values were calculated for each of the concentration ratios and for the pH by simply averaging the values within one group. The results are presented in table 52. Since these data will later be used for the calculation of the amount of ion that is bound in a complex type manner (chapter VIII), we have estimated the SE of the mean for the concentration ratio of each ion.

For the equilibrium dialysis solution-serum we proceeded as follows. On the assumption that within the pH range 6.60 to 7.75 a rectilinear correlation exists between pH and the mean value of the concentration ratio of the different ions we have calculated for  $\text{Na}^+$ ,  $\text{K}^+$  etc. the mean value to be expected at the pH value of each individual experiment from the mean values given in table 52 for pH 6.91 and 7.46 respectively. We then calculated for every individual value of  $R_{\text{Na}}$ ,  $R_{\text{K}}$  etc. the deviation from the mean at the particular pH value. Knowing these we could calculate for each of the concentration ratios the SD of an individual value and the SE of the mean for the pH ranges 6.60-7.20 and 7.20-7.75. This manner of calculating the spread is rather crude and in the case of  $R_{\text{Ca}}$  the assumption of a rectilinear correlation with pH is only correct for the

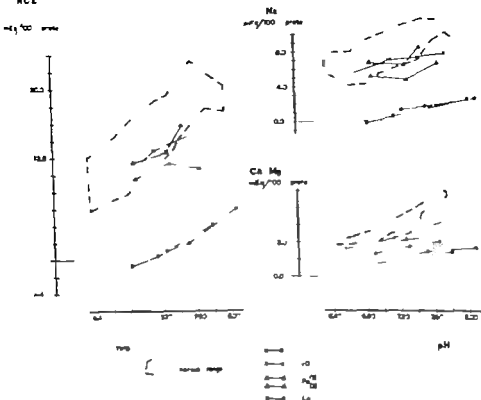


Fig. 53. Serum from patients. Correlation between pH and respectively NCE,  $\Delta$  Na,  $\Delta$  Ca + Mg (values not corrected for systematic errors). The area selected as normal range includes  $\pm 2$  SE for the individual points shown in Figs. 49 and 50.

## PURIFIED PROTEIN FRACTIONS

### DATA CONCERNING PREPARATIONS

The first experiments were done with bovine albumin and one sample of human placental  $\gamma$ -globulin, but towards the end of our investigations we have also studied albumin and  $\gamma$ -globulin prepared from human serum. The following preparations were used

#### Albumin

*Bovine plasma albumin* (Povset). The fractions are prepared from citrate plasma. After precipitation of globulins (ethanol 40%, pH 5.8), the pH of the supernatant is changed to 5.2. The precipitate which then forms contains albumin,  $\alpha$ - and  $\beta$ -globulins. After dissolving the precipitate in water  $\alpha$ - and  $\beta$ -globulins are precipitated (10% ethanol, pH 4.5–4.7) and from the supernatant albumin is finally precipitated (40% ethanol, pH 4.5). The paste is redissolved in water, stabilized and concentrated. During this stage of the procedure, new precipitate often forms (probably  $\alpha$ -globulins), which is removed. The resulting solution is freed from electrolytes by passing it over an anion-exchanging resin, then over a carbon-exchanging resin, after which the pH of the solution

*Clinical data* In two patients (Em, La) the diagnosis multiple myeloma was proven beyond doubt (histology of bone marrow, roentgenologic picture of bone lesions, paper electrophoresis of serum) and in both the paraprotein was a  $\gamma$ -globulin. In the case of patient La this was confirmed by immune electrophoresis. The third patient (Po) – courtesy of Dr J Gerbrandy and Dr S. T Boen – presented with a fairly typical clinical picture and a large amount of polymorphic plasma cells in the bone marrow but no bone lesions were seen roentgenologically. The serum contained 130 g/L protein, 80% of which was present as a typical  $\gamma$ -myeloma band on the electrophoresis strip. With the Majoor technique the abnormal protein was found in the  $\alpha_2$ -globulin fraction. Finally in the fourth patient (Vo) – courtesy of Dr N Lubsen – the clinical picture and the laboratory findings, including needle biopsy from a solitary swollen lymph gland, suggested carcinosarcoma, primary tumor unknown. In this case a pathological increase in  $\beta_2$ M-globulin was found with the aid of immuno-electrophoresis (Dr F Peetoom) the other immuno-globulins showing a decrease in concentration. On the strength of this finding the abnormality was classified as a macro-globulinaemia. With the Majoor technique the pathological protein was found to behave as a pseudo-globulin, whereas with paper electrophoresis the fraction was found in the place of the  $\beta_2$ -globulins. This combination of findings obtained with immuno-electrophoresis, paper electrophoresis and the method of Majoor remained unaltered during the further course of the disease. Dr F Peetoom commented upon the peculiar behaviour of the pathological protein since in his experience  $\beta_2$ M-globulin, contrary to  $\beta_2$ A-globulin, never behaves as a pseudo-globulin.

## RESULTS

These are listed at the end of table 47 in the same way as the results obtained for normal subjects

### NCE and $\Delta$ cation

From fig. 53 it is evident that in as far as NCE and  $\Delta$  Na are concerned, the four patients studied may be divided into two groups: the two patients with an unequivocal  $\gamma$  myeloma having very low and at pH 6.80 even zero values, the patient with macroglobulinaemia and the patient with probable  $\gamma$  myeloma having normal to low normal values. For  $\Delta$  Ca + Mg the differences are less pronounced. For  $\Delta$  K (not reproduced in fig. 53) the findings are on the whole comparable with those for  $\Delta$  Na.

### Concentration ratios

In the case of the two patients with typical  $\gamma$  myeloma we found that whereas  $R_x$  becomes 1 000 at about pH 7.00  $R_{Cl}$  becomes higher than 1 000 – in all probability because NCE tends to become negative below that pH and the net charge of the serum proteins therefore positive. A rather interesting finding is the fact that  $R_{Ca, Mg}$  remains considerably below 1 000 even at pH values of about 7.00. For the sera from the two other patients the differences in regard to normal sera are only slight.

The small number of sera notwithstanding, two points emerge from these experiments. Apparently patients who clinically and according to routine paper electrophoresis are classified as cases of  $\gamma$  myeloma can differ considerably in the cation binding properties of their proteins (Po as against patients Em and La). But in general the dialysis experiments confirm the *in vivo* ultrafiltration finding that the paraproteins of a  $\gamma$  myeloma have a low value for NCE.

A significant amount of Ca + Mg is still bound by serum protein at a pH where both the behaviour of the univalent cations and the value found for NCE indicates that protein net charge has become zero or even positive.

## PRESENTATION OF RESULTS

The results of the individual experiments are presented in table 55 which was constructed in the same manner as table 47

In the first two series of experiments in which human albumin and  $\gamma$ -globulin from the Red Cross Laboratory were dialysed the dialysis solution contained about 1-2 mEq/L citrate, and both in the dialysis solution and in the protein solution 1 to 2 mEq/L  $\text{Ca} + \text{Mg}$  must therefore have been present as a citrate-complex. Although the decrease in ionised  $\text{Ca} + \text{Mg}$  should also influence the values obtained for NCE in the sense that the latter become somewhat smaller it is unlikely that this error was more than 0.5 mEq/100 g protein (compare  $\Delta \text{Ca} + \text{Mg}$  for the experiments with and without citrate).

In table 55 one series of experiments is listed in which Na and K were determined after deproteinisation (6/ trichloroacetic acid 1:21). The experiment is included because of the results obtained for  $\Delta \text{Ca} + \text{Mg}$ .

Finally the experiment listed at the bottom of table 55 represents a special case and will be discussed in one of the following sections.

**Experiments concerning interaction between albumin and  $\gamma$ -globulin in the physiological pH range - Its possible effect on the determination of NCE and  $\Delta$  cation**

In chapter I and in the discussion of the *in vivo* ultrafiltration method we already mentioned the possibility that protein-protein interaction might occur during haemoconcentration and render difficult the interpretation of the results obtained. Since the IEP of the various plasma proteins differs considerably it is conceivable that part of the negative charges on albumin are neutralised by positive charges on the  $\gamma$ -globulin molecules. This in itself should not affect our calculation of the NCE of plasma proteins considered as a whole, provided the degree of protein-protein interaction remains proportionally the same during haemoconcentration. Since (in a solution containing various protein fractions) we can only determine the net ion equivalency for all proteins together the value found for NCE will be the same whether part of the charge on protein molecules is neutralised by oppositely charged sites on other protein molecules or whether both are neutralised by filtrable cations and anions. However this is only true if the charged sites on the molecules act in regard to each other in the same manner as in regard to the filtrable ions. If for instance, loose aggregates would form with concomitant change in the structural arrangement of ionised and non-ionised groups on the protein surface, the net charge of the aggregate might conceivably differ from the sum of the net charges of each of its components. In that case any extrapolation from values obtained at one total protein concentration to those to be expected at another total protein concentration is bound to be erroneous and so would the prediction of NCE of a protein mixture from the values obtained for the component parts.

The latter possibility is open to investigation. We have proceeded in the following manner. Solutions of albumin, of  $\gamma$ -globulin and of a mixture of both were dialysed in one container. The various protein solutions were therefore exposed to identical conditions. In table 56 the values calculated for the mixtures of albumin and  $\gamma$ -globulin (from their relative concentrations and from the values found for each protein fraction separately) are compared with the values actually found.

III between 4.5-4.8 and the conductivity between 50,000-90,000  $\Omega$ . The solution is finally freeze dried (data kindly supplied by Dr G G A. Mastenbroek)

We received the albumin in a 10% aqueous solution freshly prepared from the freeze-dried stock substance and sterilised by passage through Seitz filters. Solutions from two batches were studied. The first (batch 110) was received 10-5-60, the second (batch 123) 5-5-61. Between experiments the solutions were stored at 6°C and no bacterial contamination could be detected even after one year storage. With paper electrophoresis the solution was found to contain albumin with only traces of the globulins.

*Human plasma albumin* (Central Laboratory of the Netherlands Red Cross Bloodtransfusion Service). At our request Dr H W Krijnen was kind enough to send us two albumin preparations.

(1) *Standard albumin preparation*, which is obtained from fraction V (cold-ethanol method) 6 by first precipitating  $\alpha$  and  $\beta$ -globulins in an ethanol-ether mixture at pH 4.5 and then precipitating albumin from the supernatant by 40% ethanol pH 4.5

(2) *Albumin extra purified* by redissolving the standard albumin preparation and then treating it for a second time with ethanol-ether at pH 4.5

Both preparations were dissolved in water, pasteurised and sent to us without delay. They were received 6-3-62, kept at -15°C for 2 weeks, then thawed at 6°C and kept at that temperature during 3 to 4 days before use. The pH of the final solution was 7.40 to 7.50, the citrate content 9-12 mM/L. With paper electrophoresis both preparations were found to contain albumin with only traces of globulin.

As a result of a misunderstanding we were not prepared for the high citrate content of the solutions, with the result that the final citrate content in the containers was in the first experiments of the order of 0.5 mM/L. For that reason we also prepared

(3) *Solution of standard albumin preparation containing less than 2 mM/L citrate*. The solution of standard albumin preparation described above was dialysed against distilled water during 24 hours at 7°C. At the end of this period the protein solution contained less than 2 mM/L citrate and in the dialysis experiment subsequently performed with this albumin solution the final citrate content in the containers must have been less than 0.1 mM/L.

*Human albumin crystallised* (Nutritional Biochemical Corporation). A commercial sample was used. It is a flaky white powder which was kept sealed and stored at 5°C until immediately before the dialysis experiment - 6 g of the powder was dissolved in 60 ml of sterile stock dialysis solution at pH 7.60, resulting in a clear fluid with a brownish tinge. With paper electrophoresis the solution was found to contain albumin and surprisingly a small amount of  $\beta$ -globulin (about 5% of total protein).

#### *Gammaglobulin*

*Human placental  $\gamma$ -globulin* (Poviet). By courtesy of Dr G G A. Mastenbroek we received a sample of dried placental  $\gamma$ -globulin, prepared by a modification of cold ethanol methods 6 and 9. A 10% stock solution was prepared by dissolving the powder in 0.9% NaCl solution. The stock solution was prepared in May 1960 and kept stored at 5°C in a sterile container. With paper electrophoresis only  $\gamma$ -globulin was found.

*Human plasma  $\gamma$ -globulin* (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service). The standard  $\gamma$ -globulin preparation was used. We received it 6-3-62 as a 7% aqueous solution containing 1 mM citrate/L. It was stored and treated as described for the albumin preparations from the same laboratory. With paper electrophoresis no other protein fraction could be detected.

*Human serum  $\gamma$ -globulin* (Serva). A commercial sample was used, marketed as  $\gamma$ -globulin hypoph. pure salt free (fraction II). It is a fine white powder which we kept sealed and stored at 5°C. Immediately before use 5 g was dissolved in 50 ml of sterile stock dialysis solution at pH 7.60, resulting in a whitish opalescent fluid. With paper electrophoresis only  $\gamma$ -globulin was found.

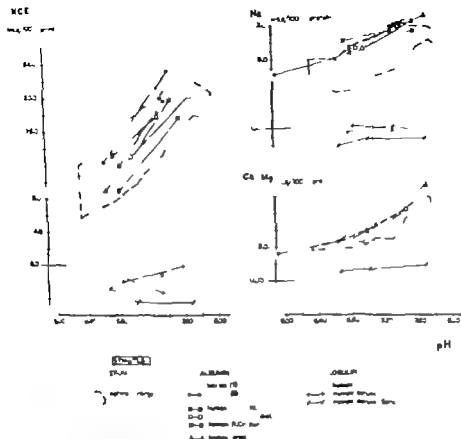


Fig 54 Albumin and  $\gamma$ -globulin. Correlation between pH and respectively NCE,  $\Delta Na$ ,  $\Delta Ca + Mg$  (values not corrected for isonormal errors). For area indicated as normal range see subscript Fig. 53.

protein content. A somewhat similar conclusion was drawn in chapter VI from the  $\gamma$ -globulin ultrafiltration results obtained in patients with an abnormal composition of serum proteins.

In an attempt to verify the above conclusion concerning the NCE for the protein fractions other than albumin and  $\gamma$ -globulin the following pilot experiment was done with the aid of Dr. Kruijsen of the Central Laboratory of the Netherlands Red Cross Bloodtransfusion Service.

In his laboratory one batch of human plasma was divided in 4 equal portions. One portion was immediately lyophilized. The other three portions were subjected to the ethanol-cold fractionation method in use at the laboratory in the following manner. On the first of these portions only the first step of the fractionation was carried out, resulting in a precipitate containing fraction I and a supernatant containing all other fractions (II-III-IV-V). The supernatant was lyophilized. For the second of the three portions the fractionation was carried one step further, resulting in a precipitate containing fraction IV and V. Again the supernatant was lyophilized. The third portion of plasma was carried through three steps and the supernatant, containing fraction V, lyophilized. It was thought that by using the supernatant only damage to the various fractions which presumably occurs most easily during precipitation could be avoided.



The relative proportion of the two fractions in the mixture was calculated in the following manner. From table 55 it can be seen that in nearly all experiments presented in table 56 albumin and  $\gamma$ -globulin were sufficiently pure to accept the  $\text{alb}/(\text{alb} + \text{euglob})$  ratio respectively  $\text{alb}/(\text{alb} + \gamma\text{-glob})$  ratio found in the mixed solutions as indicative of the true relative proportions. The fact that with the method of Majoor the mixed solutions appear to contain a much larger amount of pseudo-globulin than is found in the pure fractions, is no objection to this procedure, as in the experiments of 23-3-62 and 26-3-62 practically identical relative proportions were calculated for albumin from the results obtained with electrophoresis and from those obtained by the method of Majoor. This indicates that albumin and  $\gamma$ -globulin contribute to pseudo-globulin in relation to their relative concentrations. (Incidentally this sudden creation of pseudo-globulin by mixing albumin and  $\gamma$ -globulin stresses the need for testing the effect of this mixing on NCE and  $\Delta$  cation calculated for total protein.)

However in the series of experiments performed on 25-10-62 the albumin used contained traces of  $\alpha$ -globulin, but a fairly large amount of  $\beta$ -globulin, whereas the  $\gamma$ -globulin was comparable in purity to the samples used in earlier experiments. In this case therefore the percentage albumin given in table 56 indicates in fact  $(\text{alb} + \alpha + \beta\text{-globulin})$  as a percentage of  $(\text{alb} + \alpha + \beta + \gamma\text{-globulin})$ .

For NCE and  $\Delta$  Na the differences between calculated and observed values are of the order of the error of determination. For  $\Delta$  Ca + Mg the values observed are on the whole somewhat higher than those calculated but in view of the cumulative errors involved the difference is of doubtful significance. We conclude that under the conditions of our experiments and in as far as the determination of NCE and protein-bound cation is concerned the protein fractions act as independent macromolecules.

## RESULTS

### NCE and $\Delta$ cation

The difference between the values obtained for albumin and for  $\gamma$  globulin is evident from fig. 54. The latter contains for NCE and  $\Delta$  Na all values given in table 55 but for  $\Delta$  Ca + Mg only those from the experiments in which no or practically no citrate was present in the dialysis solution.

According to the results obtained for NCE and  $\Delta$  Na,  $\gamma$ -globulin is positively charged at physiological pH, with a net anion equivalency of about 1-2 mEq/100 g protein (in the graph expressed as a negative value for NCE). In accordance with this the pH was generally higher in the  $\gamma$  globulin solution than in the dialysis solution. At the same time however in all experiments performed with  $\gamma$  globulin Ca and Mg were found to be bound in no uncertain manner.

For the albumin fractions the most important finding is that for all preparations studied the value of NCE was within the same range as the values found for normal serum whereas  $\Delta$  Na and  $\Delta$  Ca + Mg were on the whole slightly higher although the Na and Ca + Mg concentrations in the dialysis solution were the same in the experiments with protein fractions and in those with serum. Two conclusions seem inescapable. The first one is that per unit weight, albumin binds more Cl and/or  $\text{HCO}_3^-$  than the mixture of protein fractions present in serum. We will return to this when discussing the concentration ratios. The second conclusion is that one or more fractions of  $\alpha_1$ ,  $\alpha_2$  and  $\beta$ -globulins must have an equal or higher NCE per unit weight than albumin. Otherwise serum could not have the same NCE per 100 g of protein as albumin, since  $\gamma$ -globulins - which apparently have a negative value for NCE - make up 15% of serum.

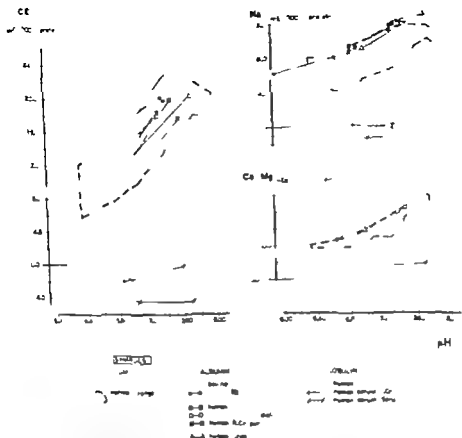


Fig 54 Albumin and  $\gamma$ -globulin. Correlation between pH and respectively NCE,  $\Delta$  Na,  $\Delta$  Ca + Mg (values not corrected for systematic errors). For area indicated as normal range see subscript fig 53

protein content. A somewhat similar conclusion was drawn in chapter VI from the *in vivo* ultrafiltration results obtained in patients with an abnormal composition of serum proteins.

In an attempt to verify the above conclusion concerning the NCE for the protein fractions other than albumin and  $\gamma$ -globulin the following pilot experiment was done with the aid of Dr. Krijnen of the Central Laboratory of the Netherlands Red Cross Bloodtransfusion Service.

In his laboratory one batch of human plasma was divided in 4 equal portions. One portion was immediately lyophilized. The other three portions were subjected to the ethanol-cold fractionation method in use at the laboratory in the following manner. On the first of these portions only the first step of the fractionation was carried out, resulting in a precipitate containing fraction I and a supernatant containing all other fractions (II-III IV V). The supernatant was lyophilized. For the second of the three portions the fractionation was carried one step further, resulting in a supernatant containing fraction IV and V. Again the supernatant was lyophilized. The third portion of plasma was carried through three steps and the supernatant, containing fraction V lyophilized. It was thought that by using the supernatant only damage to the various fractions which presumably occurs most easily during precipitation could be avoided.

The relative proportion of the two fractions in the mixture was calculated in the following manner. From table 55 it can be seen that in nearly all experiments presented in table 56 albumin and  $\gamma$ -globulin were sufficiently pure to accept the  $\text{alb}/(\text{alb} + \text{euglob})$  ratio, respectively  $\text{alb}/(\text{alb} + \gamma\text{-glob})$  ratio found in the mixed solutions as indicative of the true relative proportions. The fact that with the method of Majoor the mixed solutions appear to contain a much larger amount of pseudo-globulin than is found in the pure fractions, is no objection to this procedure, as in the experiments of 23-3-62 and 26-3-62 practically identical relative proportions were calculated for albumin from the results obtained with electrophoresis and from those obtained by the method of Majoor. This indicates that albumin and euglobulin contribute to pseudo-globulin in relation to their relative concentrations. (Incidentally this sudden creation of pseudo-globulin by mixing albumin and  $\gamma$ -globulin stresses the need for testing the effect of this mixing on NCE and  $\Delta$  cation calculated for total protein.)

However in the series of experiments performed on 25-10-62 the albumin used contained traces of  $\gamma$ -globulin, but a fairly large amount of  $\beta$ -globulin, whereas the  $\gamma$ -globulin was comparable in purity to the samples used in earlier experiments. In this case therefore the percentage albumin given in table 56 indicates in fact  $(\text{alb} + \alpha + \beta\text{-globulin})$  as a percentage of  $(\text{alb} + \alpha + \beta + \gamma\text{-globulin})$ .

For NCE and  $\Delta$  Na the differences between calculated and observed values are of the order of the error of determination. For  $\Delta$  Ca + Mg the values observed are on the whole somewhat higher than those calculated but in view of the cumulative errors involved the difference is of doubtful significance. We conclude that under the conditions of our experiments and in as far as the determination of NCE and protein bound cation is concerned the protein fractions act as independent macromolecules.

## RESULTS

### NCE and $\Delta$ cation

The difference between the values obtained for albumin and for  $\gamma$ -globulin is evident from fig. 54. The latter contains for NCE and  $\Delta$  Na all values given in table 55 but for  $\Delta$  Ca + Mg only those from the experiments in which no or practically no citrate was present in the dialysis solution.

According to the results obtained for NCE and  $\Delta$  Na,  $\gamma$  globulin is positively charged at physiological pH with a net anion equivalency of about 1-2 mEq/100 g protein (in the graph expressed as a negative value for NCE). In accordance with this the pH was generally higher in the  $\gamma$  globulin solution than in the dialysis solution. At the same time however in all experiments performed with  $\gamma$ -globulin Ca and Mg were found to be bound in no uncertain manner.

For the albumin fractions the most important finding is that for all preparations studied the value of NCE was within the same range as the values found for normal serum, whereas  $\Delta$  Na and  $\Delta$  Ca + Mg were on the whole slightly higher although the Na and Ca + Mg concentrations in the dialysis solution were the same in the experiments with protein fractions and in those with serum. Two conclusions seem inescapable. The first one is that, per unit weight, albumin binds more Cl and/or  $\text{HCO}_3^-$  than the mixture of protein fractions present in serum. We will return to this when discussing the concentration ratios. The second conclusion is that one or more fractions of  $\alpha_1$ ,  $\alpha_2$  and  $\beta$ -globulins must have an equal or higher NCE per unit weight than albumin. Otherwise serum could not have the same NCE per 100 g of protein as albumin since  $\gamma$  globulins - which apparently have a negative value for NCE - make up 15% of serum.

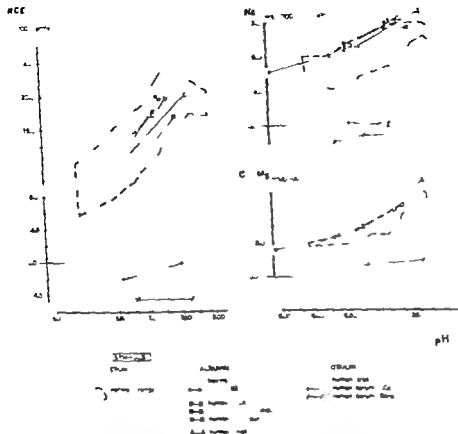


Fig 54 Albumin and  $\gamma$ -globulin. Correlation between pH and respectively NCE,  $\Delta$  Na,  $\Delta$  Ca,  $\Delta$  Mg (values not corrected for systematic errors). For area indicated as normal range see subscript fig 53

protein content. A somewhat similar conclusion was drawn in chapter VI from the *in vitro* ultrafiltration results obtained in patients with an abnormal composition of serum proteins.

In an attempt to verify the above conclusion concerning the NCE for the protein fractions other than albumin and  $\gamma$ -globulin the following pilot experiment was done with the aid of Dr. Kroon of the Central Laboratory of the Netherlands Red Cross Bloodtransfusion Service.

In his laboratory one batch of human plasma was divided in 4 equal portions. One portion was immediately lyophilized. The other three portions were subjected to the ethanol-cold fractionation method in use at the laboratory in the following manner. On the first of these portions only the first step of the fractionation was carried out, resulting in a precipitate containing fraction I and supernatant containing all other fractions (II-III, IV, V). The supernatant was lyophilized. For the second of the three portions the fractionation was carried one step farther, resulting in supernatant containing fraction IV and V. Again the supernatant was lyophilized. The third portion of plasma was carried through three steps and the supernatant, containing fraction V lyophilized. It was thought that by using the supernatant only, damage to the various fractions which presumably occurs most easily during precipitation, could be avoided.

The 3 sterile flasks, containing the supernatant of successive steps in the fractionation procedure in a lyophilized state, were stored at  $-15^{\circ}\text{C}$  for 4 weeks. Then their contents were redissolved in an amount of distilled water calculated to bring the final protein concentration in each flask to  $\pm 70 \text{ g/L}$ . Following this the contents of each flask were dialysed against distilled water for 36 hours (at  $30^{\circ}\text{C}$  and under sterile conditions) to decrease the rather high concentration of citrate. Immediately thereafter equilibrium dialysis was performed in the usual manner at pH 7.50 with the contents of the 3 flasks (containing fractions II III IV-V fractions IV V and fraction V respectively). The results are shown at the bottom of table 55. They are marred by the fact that obviously citrate was still present in significant amounts [(cal min  $\text{Cl} + \text{HCO}_3$ ) for dialysis solution 4.9 values for  $\Delta \text{Ca} + \text{Mg}$  lower than expected]. Furthermore, whereas the solution containing fractions IV-V and the solution containing fraction V were only slightly opalescent the solution containing fractions II III IV-V showed flocculation.

Nevertheless it would appear that from the results some conclusions can be drawn. Whereas in the case of fractions II-III IV-V (which should be very much like plasma) the values for NCE,  $\Delta \text{Na}$  and  $\Delta \text{K}$  are indeed of the same magnitude as found for normal serum, the values found for fractions IV-V and for fraction V are higher especially those for  $\Delta \text{Na}$  and  $\Delta \text{K}$  and ~ in the case of fraction V - also that for NCE. The electrophoretic strip showed that fractions IV V were composed of albumin,  $\alpha_1$ ,  $\alpha_2$  and  $\beta$ -globulin in about the same relative proportions as in serum, whereas fraction V contained albumin with some  $\alpha_2$ -globulin and a trace of  $\alpha_1$ -globulin. We must therefore conclude that either  $\alpha_2$ -globulin has a NCE which per unit weight is about 8 times larger than the NCE found for albumin, or that during the final precipitation the net negative charge of albumin is considerably altered. The experiment should obviously have included equilibrium dialysis of the albumin obtained from fraction V but such as it is the experiment can serve to illustrate the need for further investigation on this point and for caution in the calculation of values for serum from values obtained with purified fractions.

Returning to the results obtained for albumin it is difficult to say whether the differences between the values found for the various preparations are real in view of the SE of the determination. An exception must be made for the experiments with the Red Cross preparations in which the citrate content of the dialysis solution was of the order of  $0.5 \text{ mM/L}$ . But even there a distinct difference is only found for  $\Delta \text{Ca} + \text{Mg}$ , the NCE value remaining relatively unaffected. The small number of experiments and the variety of preparations studied effectively prevents an accurate estimate of the pH effect for albumin and for  $\gamma$ -globulin although for albumin the relation with pH appears to be similar to that found for normal serum. Table 57 may serve to indicate the approximate values at pH 7.45. These were obtained by simply averaging all values available within the pH range 7.30-7.70 with the exception of

for NCE,  $\Delta \text{Na}$   $\Delta \text{K}$  the experiment in which the serum was deproteinised

for  $\Delta \text{Ca} + \text{Mg}$  the experiments in which a significant amount ( $> 0.1 \text{ mM/L}$ ) of citrate was present in the dialysis solution

In table 57 the average values have not been corrected for systematic errors and the values listed for normal serum therefore refer also to uncorrected results (For  $\Delta \text{K}$  the normal value at pH 7.45 was estimated from fig. 50)

When we attempt to correct for the systematic errors in NCE and  $\Delta \text{Na}$ , we meet with the difficulty that in the case of the purified protein fractions the flame photometer error is not known for certain. The evidence presented in fig. 12 (chapter III) suggests that in the case of albumin the necessary correction may be about  $0.8 \text{ mEq/100 g protein}$ , and 1 in the case of  $\gamma$ -globulin zero. But this conclusion is only based on one experiment and in equilibrium dialysis experiments with albumin comparison of the distribution of  $^{23}\text{Na}$  (as measured with a scintillation counter) with that of  $^{23}\text{Na}$  (as measured by flame photometry) suggested that in the case of albumin the flame photometer error is not larger than in the case of serum. No ashing experiments were done with salt solutions containing albumin or  $\gamma$ -globulin.

From the evidence available at present we can only estimate that in the case of  $\Delta Na$  and of NCE the correction for the flame photometer error is somewhere between 0.2 and 0.8 mEq/100 g albumin and between 0.0 and 0.2 mEq/100 g  $\gamma$ -globulin. In the case of NCE the correction for carbamino-protein should be added which we have assumed to be equal to the correction accepted for normal serum, i.e. 0.3 mEq/100 g albumin or  $\gamma$ -globulin. According to this reasoning, the corrected values for NCE should therefore be 20.2 to 20.8 mEq/100 g albumin and -1.9 to -1.7 mEq/100 g  $\gamma$ -globulin. The magnitude of the correction remains small and does not affect the conclusions concerning the different behaviour of albumin and  $\gamma$ -globulin and the relatively low value of NCE in the case of albumin.

In comparing the values given for  $\Delta Na$ ,  $\Delta K$  and  $\Delta Ca + Mg$  it should once again be kept in mind that they are not corrected for the effect of the Donnan equilibrium. Since the latter depends on net charge the differences between the values obtained for albumin and for  $\gamma$ -globulin will in reality be larger.

#### Concentration ratios

These are presented in table 55, but we will only consider  $R_x$  and  $R_{Cl}$  in more detail. To facilitate comparison with the results obtained for normal serum we have averaged our results for the two pH ranges 6.60-7.20 and 7.20-7.70, as in table 52 for normal sera. (All experiments have been included with the exception of the one in which Na was determined after deproteinisation).

Table 58 summarises our efforts. The effect on  $R_x$  of the correction for the estimated flame photometer error discussed in the previous paragraph is indicated. In the case of albumin and  $\gamma$ -globulin we have not attempted to calculate the SE of the mean as we are dealing here with fractions prepared from different sources and by different methods. But even so it would appear that for albumin  $R_x$  is definitely lower and  $R_{Cl}$  somewhat higher than for normal serum. Since for albumin the negative net charge is certainly somewhat larger per unit weight than for the proteins as present in serum, this discrepancy between the concentration ratios of the two oppositely charged ions is compatible with the assumption that albumin binds per unit weight a larger amount of Cl in a complex-type manner than the other serum proteins. This explains to some extent the fact that the difference between serum and albumin as regards NCE is less than the differences found for  $\Delta Na$  and  $\Delta Ca + Mg$  would lead us to expect.

For  $\gamma$ -globulin the number of samples is so small that no definite conclusion is possible whether the difference between  $R_x$  and  $R_{Cl}$  is a real one here we cannot decide. But the fact that they are both higher than 1.000 is another indication that the net charge of  $\gamma$ -globulin is positive in the pH range studied and gives even more significance to the fact that  $R_{Cl} \times$  is still 0.84 at pH 7.47 and 0.91 at pH 6.82 (see table 55).

## CHAPTER VIII

### *Comparison and discussion of the results obtained by means of in vivo ultrafiltration and by means of equilibrium dialysis*

We will first consider the values obtained for NCE, then the values obtained for protein bound cation and finally we will try to estimate values for the Donnan ratio and for the amounts of ion bound in a salt type respectively complex type manner

#### NET CATION EQUIVALENCY (NCE)

##### RESULTS OBTAINED FOR NORMAL SUBJECTS

Table 59 compares the mean value of NCE obtained by means of *in vivo* ultrafiltration (table 42, chapter VI) with that obtained by means of equilibrium dialysis of serum samples (table 48, chapter VII). When we assume the buffer value to be 10.3 mEq/100 g protein/unit pH (see below) we find that at pH 7.34 the difference between the two mean values is 0.4 mEq/100 g protein. For the *not corrected* values of NCE in the 28 *in vivo* ultrafiltration experiments the SE of the mean was calculated as 0.2 mEq/100 g protein (see Addendum). Since a correction for systematic errors increases the accidental error we can conclude that the difference between the two mean values given in table 59 is *not statistically significant*.

In the case of the 9 *in vivo* ultrafiltration experiments at the end of which serum was obtained for dialysis the results can be compared with those obtained in the paired dialysis experiments (table 60). After correction for systematic errors the mean value of NCE is somewhat lower for the *in vivo* ultrafiltration series but again the difference is too small to be significant. The latter conclusion is confirmed when the pairs of experiments are considered separately (last column of table 60).

The influence of differences in pH was eliminated by calculating for the dialysis experiments the NCE value to be expected at the pH of the corresponding *in vivo* ultrafiltration experiments assuming a buffer value of 10.3 mEq/100 g protein/unit pH.

When we add to the 9 pairs of experiments in which serum was dialysed the 4 pairs of experiments in which plasma was dialysed, the mean value of NCE becomes 17.3 (at pH 7.35) in the case of the *in vivo* ultrafiltration experiments against 18.0 (at pH 7.34) in the case of the dialysis experiments. The difference is now somewhat larger due to the fact that for the dialysis experiments the mean value has become somewhat higher. Although not statistically significant, the observed value is compatible with the existence of a small positive error in NCE due to the amount of heparin present in the plasma samples.

When we average the two mean values of NCE presented in table 59 while taking into account the number of subjects studied in each of the two series of experiments and the

observed relation between pH and NCE (see below), we obtain the following estimate for the average value of the NCE of normal plasma proteins

$$\text{NCE} = 17.4 \text{ mEq/100 g protein (at pH 7.35)}$$

or

$$\text{NCE} = 12.2 \text{ mEq/L plasma (at pH 7.35)}$$

when we consider normal plasma to contain 70 g protein per litre.

The fact that for the *in vivo* ultrafiltration experiments the 'weighted' mean of NCE values not corrected for systematic errors (presented in the Addendum) is 0.2 mEq/100 g lower than their arithmetic mean value, on which the corrected mean value given in table 59 is based, indicates that by neglecting the different 'weight' of the individual *in vivo* ultrafiltration experiments we overestimate the mean value of NCE by about 1% at most.

For the calculation of the correlation between pH and NCE the results of the dialysis experiments are available. If we adjust the equations derived in chapter VII to the value accepted as the best estimate of NCE for normal plasma proteins at pH 7.34 (see above) we obtain the following two equations for the pH range 6.60 to 7.00

$$\text{NCE (mEq/100 g protein)} = 10.0 (\text{pH} - 5.64)$$

for the pH range 7.00 to 7.70

$$\text{NCE (mEq/100 g protein)} = 10.7 (\text{pH} - 5.72).$$

The reader is reminded of the fact that the difference between the two equations is due to the assumption that carbamino-protein is present at pH values above 7.00.

The results of our experiments indicate that in the pH range observed in clinical medicine a reasonable estimate of the value of NCE at a given pH can be obtained from the following equation

$$\text{NCE (mEq/100 g protein)} = 10.3 (\text{pH} - 5.66)$$

provided the composition of plasma proteins is normal and the concentrations of the plasma electrolytes do not differ much from normal.

We have not calculated the SE for the mean values given above. The correction for systematic errors precludes this. However the magnitude of the spread observed in our experiments is indicated by the fact that, for both methods used and at any given pH all experimental values for NCE were within  $\pm 4$  mEq of the non-corrected mean value calculated for that particular pH value.

#### RESULTS OBTAINED FOR PATIENTS AND RESULTS OBTAINED WITH PURIFIED PROTEIN FRACTIONS

Our observations suggest that it is unlikely that factors other than pH and protein composition have an important effect on the magnitude of NCE (expressed per 100 g protein).



*Comparison and discussion of the results  
obtained by means of in vivo ultrafiltration and by means  
of equilibrium dialysis*

We will first consider the values obtained for NCE, then the values obtained for protein-bound cation and finally we will try to estimate values for the Donnan ratio and for the amounts of ion bound in a salt type respectively complex type manner

### NET CATION EQUIVALENCY (NCE)

#### RESULTS OBTAINED FOR NORMAL SUBJECTS

Table 59 compares the mean value of NCE obtained by means of *in vivo* ultrafiltration (table 42, chapter VI) with that obtained by means of equilibrium dialysis of serum samples (table 48 chapter VII) When we assume the buffer value to be 10.3 mEq/100 g protein/unit pH (see below) we find that at pH 7.34 the difference between the two mean values is 0.4 mEq/100 g protein. For the *not corrected* values of NCE in the 28 *in vivo* ultrafiltration experiments the SE of the mean was calculated at 0.2 mEq/100 g protein (see Addendum). Since a correction for systematic errors increases the accidental error we can conclude that the difference between the two mean values given in table 59 is not statistically significant.

In the case of the 9 *in vivo* ultrafiltration experiments at the end of which serum was obtained for dialysis, the results can be compared with those obtained in the paired dialysis experiments (table 60). After correction for systematic errors the mean value of NCE is somewhat lower for the *in vivo* ultrafiltration series, but again the difference is too small to be significant. The latter conclusion is confirmed when the pairs of experiments are considered separately (last column of table 60).

The influence of differences in pH was eliminated by calculating for the dialysis experiments the NCE value to be expected at the pH of the corresponding *in vivo* ultrafiltration experiments assuming a buffer value of 10.3 mEq/100 g protein/unit pH.

When we add to the 9 pairs of experiments in which serum was dialysed the 4 pairs of experiments in which plasma was dialysed, the mean value of NCE becomes 17.3 ( $\pm$  pH 7.35) in the case of the *in vivo* ultrafiltration experiments against 18.0 ( $\pm$  pH 7.34) in the case of the dialysis experiments. The difference is now somewhat larger due to the fact that for the dialysis experiments the mean value has become somewhat higher. Although not statistically significant, the observation is compatible with the existence of small positive error in NCE due to the amount of heparin present in the plasma samples.

When we average the two mean values of NCE presented in table 59 while taking into account the number of subjects studied in each of the two series of experiments and the

in which the pH was within the range 7.10-7.50. The Na value is corrected for the error due to flame photometry (table 50 in chapter VII). Finally we have listed the values obtained after correcting the results of the dialysis experiments for the Donnan effect. The manner in which the latter correction was made needs some explanation.

In chapter I it was shown that in order to obtain the total amount of cation bound in one way or other by protein an amount equal to  $(1 - r) [\text{Cat}]_0$  (for bivalent cations  $(1 - r^2) [\text{Cat}]_0$ ) must be added to  $\Delta \text{Cat}$ . In these equations, both  $\Delta \text{Cat}$  and  $r$  refer to the actual protein concentration found in the experiments. From evidence presented in the third part of this chapter we can estimate the Donnan ratio to be 0.964 at pH 7.33 for normal serum with protein concentration of 70 g/Kg H<sub>2</sub>O. For the 14 dialysis experiments from which the data in table 61 were derived the average protein concentration was 62.3 g/Kg H<sub>2</sub>O and since the relation between protein concentration and  $r$  is practically linear one for the electrolyte concentrations in our experiments (see chapter I) we can estimate the average value of  $r$  to be 0.968 at the protein concentrations observed in our experiments. From this, and from  $[\text{Cat}]_0$ , we calculate that the concentration of filtrable cation opposing filtrable anion inside the dialysis unit lower than outside by 4.6 mEq/Kg H<sub>2</sub>O in the case of Na, and by 0.26 mEq/Kg H<sub>2</sub>O in the case of Ca + Mg. These values must therefore be subtracted from  $[\text{Cat}]_0$  to obtain the concentration of 'free' cation in the serum. The cation not bound by protein in one way or other. Conversely to obtain the total amount of cation protein-bound at the protein concentration of 62.3 g/Kg H<sub>2</sub>O the above values must be added to the values of  $\Delta \text{Cat}$  actually found. The values thus corrected must still be multiplied by 100/62.3 to obtain the value for  $\Delta \text{Cat}$  in mEq per 100 g of protein.

In this way the values listed on the last line of table 61 were calculated. Although an approximation they can serve to indicate the magnitude of the Donnan effect.

If we compare the corrected dialysis results with the result of the *in vivo* ultrafiltration experiments we find that the latter are about 4.8 mEq/100 g protein lower in the case of protein-bound Na and about 0.15 mEq/100 g protein lower in the case of protein-bound Ca + Mg. In view of the fact that we found nearly the same value for NCE with both methods we can assume that the differences observed for protein bound Na and for protein-bound Ca + Mg are indeed due to neglect of the change in Donnan ratio occurring during haemoconcentration. It should be noted that these differences are of the same magnitude as the error estimated in chapter IV from the trend of the sum concentration of  $\text{Cl} + \text{HCO}_3$  during haemoconcentration assuming 2 mEq of Cl to be complex-bound by the albumin normally present in one litre of plasma. This finding supports the assumption that in plasma a certain amount of anion is bound in a complex type manner by protein. It is also compatible with a Donnan ratio of about 0.97 at the capillary membrane (when pH and protein concentration are normal).

For K no values are given in table 61. In the *in vivo* ultrafiltration experiments no reliable results could be obtained and the amount calculated from the dialysis experiments is dubious because of the relatively large correction necessary for the flame photometer error. But on the average about 0.3 to 0.4 mEq of K must have been bound per 100 g normal plasma protein at normal pH values.

We can make an estimate of the influence of pH on the total amount of protein-bound Na and protein-bound Ca + Mg by correcting the experimental relation found for  $\Delta \text{Na}$ , and for  $\Delta \text{Ca} + \text{Mg}$  with the aid of the values which the Donnan ratio is estimated to have at pH 6.91 and 7.46 (third part of this chapter) while using the same approach as described above in connection with table 61.

After correcting for flame photometer error and for the Donnan effect, the following

The effect of pH is evident from the discussion in the previous section. As a result one can expect the value of NCE to vary from about 8 mEq/100 g protein at pH 6.90 to a value of about 23 mEq/100 g protein at pH 7.70 – the pH values mentioned representing the extremes of the clinical pH range.

As to protein composition the results both of the *in vivo* ultrafiltration experiments and of the dialysis experiments with pathological sera and purified protein fractions indicate that for normal  $\gamma$  globulin and for the paraprotein(s) of  $\gamma$ -myeloma the NCE is of the order of 2-6 mEq/100 g protein at a pH value of 7.35. For the other fractions our results are more equivocal but it seems reasonable to conclude that *in vivo* albumin and at least some of the  $\alpha$  and possibly the  $\beta$ -globulins have a NCE approximately 10 to 20% higher than that found for the normal mixture of plasma proteins, the value being on the average 20-21 mEq/100 g protein at a pH value of 7.35. This is a surprisingly low value for albumin: preliminary experiments in which fractions obtained with the cold-ethanol method 6 were compared (chapter VII) seem to indicate that in the process of precipitation and further purification the NCE of albumin decreases. Another possibility is that some of the  $\alpha$  and  $\beta$ -globulins have an excessively high NCE (40-100 mEq/100 g protein).

In the *in vivo* ultrafiltration experiments it was observed that the  $\beta$ -type of myeloma is different from the  $\gamma$  type in that its paraproteins have a much higher NCE. But the fact that with equilibrium dialysis the serum proteins of one patient with a  $\gamma$ -type of myeloma were found to have a nearly normal value for NCE indicates that the paper electrophoretic pattern is not an infallible guide where the magnitude of NCE is concerned.

Finally it must be stressed that even in patients with severe uraemia NCE of the plasma proteins was in the range expected when the effect of pH and protein composition was taken into account. This is interesting in view of the fact that in these patients relatively large amounts of organic substances are present in plasma. In the 3 patients with hypercholesterolaemia also a normal value was observed for NCE.

## QUANTITY OF PROTEIN BOUND CATION

### RESULTS OBTAINED FOR NORMAL SUBJECTS

The values calculated from the *in vivo* ultrafiltration experiments and from the dialysis experiments are both underestimates because of the effect of the Donnan equilibrium. A correction can be made if the Donnan ratio is known. In the third part of this chapter we will make an attempt to estimate from our dialysis experiments average values for the Donnan ratio at different pH values. Since we possess no direct information concerning the composition of interstitial fluid no correction is possible for the results obtained with *in vivo* ultrafiltration. But by comparing the latter with the values found when the results of the dialysis experiments are corrected for the Donnan effect, some conclusions concerning the Donnan ratio at the capillary membrane should be possible.

In table 61 we have first listed the results of the *in vivo* ultrafiltration experiments as presented in table 41 (chapter VI). The values are those obtained after correction for systematic errors (with the exception, of course, of the error assumed for the Donnan effect). Below these are listed the results obtained for serum in the 14 dialysis experiments

In chapter VII. If an approximate correction is made for the Donnan effect with the aid of the Donnan ratios estimated in the third part of this chapter we arrive at the conclusion that at pH 7.40 total albumin-bound Na is about 22 mEq/100 g albumin, whereas at the same pH the amount of Na bound by  $\gamma$ -globulin is negligible, or even negative - indicating that in the latter case protein net charge must be positive. In a similar manner we can estimate that at pH 7.40 total protein-bound Ca + Mg is approximately 5.0 mEq/100 g albumin and about 1.0 mEq/100 g  $\gamma$ -globulin when the concentration of 'free' cation is about 2.6 mEq/Kg  $H_2O$  for Ca, and 1.3 mEq/Kg  $H_2O$  for Mg.

The behaviour of K towards albumin and  $\gamma$ -globulin is qualitatively the same as that of Na.

## THE DONNAN RATIO AND THE NATURE OF ION-PROTEIN BINDING - NEGATIVE NET CHARGE OF PROTEIN

In chapter I we argued that in dialysis experiments such as ours the Donnan ratio can be obtained either from the concentration ratio of a given cation (or anion) when the magnitude of its complex-bound fraction is known, or from the concentration of filtrable anion and the protein net charge in the serum. But neither approach is possible in our case since we determine NCE, not protein net charge. The latter can only be calculated from the first if the total amounts of both complex-bound cation and anion are known and for these we must know the Donnan ratio. We are therefore caught in a cleft stick. However we can try how far reasoning will lead us.

### NORMAL SERUM

#### Estimation of Donnan ratio

In chapter I it was shown that Cl is the only one of the ions under consideration for which a reasonable estimate can be made of the quantity which at physiological pH is complex-bound by albumin. Furthermore, evidence was presented which indicates that at pH 7.00 the amount of Na bound in a complex-type manner by albumin must be negligible, i.e. presumably less than 2% of the Na present in the solution.

In our dialysis experiments we found, both with serum and with albumin and for the whole pH range studied,  $R_{Na}$  lower than  $R_{Cl}$ . The difference appears to be real since it was found in every single experiment. The difference is the more pronounced the lower the pH. Therefore, one or both of these ions must be partially complex bound by protein. The fact that both for serum and for albumin the average value of  $R_{Cl}$  was found very nearly 1.000 at a pH where NCE is still about 10 mEq per 100 g of protein suggests that around pH 7.00 it must be mainly Cl which is bound in a complex-type manner. Qualitatively therefore both our findings for normal serum and for albumin are in agreement with the experimental results obtained by SCATCHARD *et al.* and by CARR, who studied purified albumin and used a different technique.

In order to interpret our data on serum in a more quantitative way we will first consider the results obtained with albumin. We assume that at pH 6.80 (the average pH of the low pH range in our experiments with albumin - see table 53 in chapter VII) no complex-bound Na exists. In that case  $R_{Na} = \infty$ . With the aid of equation (9) in chapter I

relationship between protein bound Na and pH is found

$$\text{protein bound Na (mEq/100 g)} = 7.3 (\text{pH} - 5.10)$$

In the case of protein-bound Ca + Mg the correction for Donnan effect somewhat increases the curvilinearity of the relation with pH. But since for the pH range 7.00 to 7.50 the relation is roughly rectilinear we find after correction for the Donnan effect

$$\text{protein bound Ca + Mg (mEq/100 g)} = 2.7 (\text{pH} - 5.87)$$

In our experiments the ratio between the concentrations of free Ca and free Mg was about 2 : 1. If we assume that the protein bound fraction is about equal for both ions in the pH range studied (see chapter VII) we can estimate the increase per unit pH to be for Ca 1.8 mEq/100 g protein and for Mg 0.9 mEq/100 g protein.

#### RESULTS OBTAINED FOR PATIENTS AND RESULTS OBTAINED WITH PURIFIED PROTEIN FRACTIONS

The error due to the Donnan effect is considerable in the case of protein-bound Na but it is relatively small in the case of the bivalent cations because in the latter case most of the binding is of the complex type and thus not affected by the Donnan ratio. For Ca and Mg some conclusions can therefore be drawn from *in vivo* ultrafiltration in patients with abnormal plasma proteins, if allowance is made for differences in free Ca and free Mg concentration. On the whole the results obtained with *in vivo* ultrafiltration and those obtained for the few pathological sera which were dialysed, are in good agreement with the results obtained when dialysing purified protein preparations. They all show that although Ca (and Mg) are certainly bound to a lesser extent by  $\gamma$  globulin than by albumin the difference is not so pronounced as for NCE. At physiological pH values  $\gamma$  globulin binds still relatively large amounts of bivalent cations although its net charge is only just negative or even slightly positive.

While discussing the results obtained for NCE we have already mentioned the observation that the serum of one patient with an apparently typical  $\gamma$  myeloma had an unexpectedly high value of NCE. The same was found for  $\Delta$  Na, but the difference was not nearly so marked for  $\Delta$  Ca + Mg.

For the various albumin solutions studied by equilibrium dialysis the spread in  $\Delta$  Na was remarkably small, much smaller indeed than the spread found for the NCE values. Furthermore since in the case of albumin both  $\Delta$  Na and  $\Delta$  Ca + Mg were found distinctly higher than in the case of normal serum the fact that the NCE values were on the whole only slightly higher than the values found for normal serum must indicate that more Cl (and  $\text{HCO}_3^-$ ) is complex bound by 100 g of albumin than by 100 g of normally composed serum proteins.

Because of the limited number of experiments we have made no attempt to derive equations for the relation between pH and NCE respectively protein-bound cation in the case of albumin and of  $\gamma$ -globulin but the general trend can be gathered from fig. 54

in chapter VII. If an approximate correction is made for the Donnan effect with the aid of the Donnan ratios estimated in the third part of this chapter we arrive at the conclusion that at pH 7.40 total albumin-bound Na is about 22 mEq/100 g albumin, whereas at the same pH the amount of Na bound by  $\gamma$ -globulin is negligible, or even negative - indicating that in the latter case protein net charge must be positive. In a similar manner we can estimate that at pH 7.40 total protein-bound Ca + Mg is approximately 5.0 mEq/100 g albumin and about 1.0 mEq/100 g  $\gamma$ -globulin when the concentration of free cation is about 2.6 mEq/Kg  $H_2O$  for Ca, and 1.3 mEq/Kg  $H_2O$  for Mg.

The behaviour of K towards albumin and  $\gamma$ -globulin is qualitatively the same as that of Na.

## THE DONNAN RATIO AND THE NATURE OF ION PROTEIN BINDING - NEGATIVE NET CHARGE OF PROTEIN

In chapter I we argued that in dialysis experiments such as ours the Donnan ratio can be obtained either from the concentration ratio of a given cation (or anion) when the magnitude of its complex-bound fraction is known, or from the concentration of filtrable anion and the protein net charge in the serum. But neither approach is possible in our case since we determine NCE, not protein net charge. The latter can only be calculated from the first if the total amounts of both complex-bound cation and anion are known and for these we must know the Donnan ratio. We are therefore caught in a cleft stick. However we can try how far reasoning will lead us.

### NORMAL SERUM

#### Estimation of Donnan ratio

In chapter I it was shown that Cl is the only one of the ions under consideration for which a reasonable estimate can be made of the quantity which at physiological pH is complex bound by albumin. Furthermore, evidence was presented which indicates that at pH 7.00 the amount of Na bound in a complex-type manner by albumin must be negligible, i.e. presumably less than 2% of the Na present in the solution.

In our dialysis experiments we found both with serum and with albumin and for the whole pH range studied,  $R_{Na}$  lower than  $R_{Cl}$ . The difference appears to be real since it was found in every single experiment. The difference is the more pronounced the lower the pH. Therefore, one or both of these ions must be partially complex-bound by protein. The fact that both for serum and for albumin the average value of  $R_{Cl}$  was found very nearly 1.000 at a pH where NCE is still about 10 mEq per 100 g of protein suggests that around pH 7.00 it must be mainly Cl which is bound in a complex-type manner. Qualitatively therefore both our findings for normal serum and for albumin are in agreement with the experimental results obtained by SCATCHARD *et al* and by CARA, who studied purified albumin and used a different technique.

In order to interpret our data on serum in a more quantitative way we will first consider the results obtained with albumin. We assume that at pH 6.80 (the average pH of the low pH range in our experiments with albumin - see table 58 in chapter VII) no complex-bound N exists. In that case  $R_X = r$ . With the aid of equation (9) in chapter I,

we calculate then from table 58 (using the lowest of the two values given for corrected  $R_{Na}$ )

complex bound  $Cl = 5.7 \text{ mEq/Kg H}_2\text{O}$  at an albumin concentration of  $70 \text{ g/Kg H}_2\text{O}$

Thus we find that at pH 6.80 and at a  $Cl$  concentration of about  $130 \text{ mEq/Kg H}_2\text{O}$   $5.7$  moles  $Cl$  are bound per mole albumin (mol weight  $\sim 70,000$ ). This should be the maximum value since it was calculated on the assumption that no  $Na$  is complex bound. If  $1/$  of the  $Na$  present in the albumin solution were complex bound, the Donnan ratio would be  $0.964$  (equation (3) in chapter I) and in that case we calculate that  $4.6$  moles  $Cl$  are bound per mole albumin. In a similar manner we calculate from the experiments in the high pH range (table 58) that, at pH 7.44  $4.6$  moles  $Cl$  are complex bound per mole albumin if no  $Na$  is complex bound, and that  $3.4$  moles  $Cl$  must be complex bound per mole albumin if  $1/$  of  $Na$  is bound in a complex type manner.

In chapter I we came to the conclusion that the data from the literature indicate that at pH 7.40 a value between  $0.5$  and  $3.0$  is the most reasonable estimate for the moles of  $Cl$  that are complex bound per mole albumin. The values calculated above are somewhat higher - especially the one calculated on the assumption that no  $Na$  is bound in a complex type manner. In this connection it must be remembered that the results obtained by SCATCHARD *et al.* by VAN OS & KOOPMAN-VAN EUPEN and by CARR do not exclude the possibility that  $1-2/$  of  $Na$  is complex-bound at our experimental conditions.

If we now return to our main interest which is *plasma under physiological conditions* we cannot neglect the observation of Carr that  $\alpha$ - and  $\beta$ -globulins bind  $Na$  in a complex type manner. His results appear to indicate that, at pH 7.50  $2-3 \text{ mEq}$  of  $Na$  are bound per  $100 \text{ g}$  of these proteins when the  $Na$  concentration in the solution is only  $15-20 \text{ mEq/L}$ . In the case of serum therefore we cannot consider  $R_x$  to be equal to  $r$ . But since albumin is apparently the only one of the serum proteins to bind  $Cl$  in a complex type manner at physiological pH (chapter I), we can make an estimate of the Donnan ratio by first calculating the amount of complex bound  $Cl$  present in serum from the albumin concentration of the latter and from the amount of  $Cl$  bound per mole albumin as calculated from the dialysis experiments.

In our experiments  $60\%$  of serum protein was albumin (average value found by paper electrophoresis, 60% that found with the method of Major, 58). If we assume that the relation between pH and complex-bound  $Cl$  is linear over the pH range 6.80 to 7.50, we can estimate the concentration of complex-bound  $Cl$  in serum at pH 6.91 and at pH 7.46 to be respectively  $3.31$  and  $2.74 \text{ mEq/Kg H}_2\text{O}$  when no  $Na$  is complex-bound by albumin, and respectively  $2.67$  and  $2.03 \text{ mEq/Kg H}_2\text{O}$  when  $1\%$  of  $Na$  is complex-bound.

With the aid of equation (5) in chapter I and the data presented in table 52 (chapter VII) we then calculate the following values for the Donnan ratio of serum

	pH 6.91	pH 7.46
if no $Na$ is complex-bound by albumin	0.9666	0.9555
if $1/$ of $Na$ is complex bound by albumin	0.9717	0.9614

A glance at the average values of  $R_x$  found for normal serum (table 53 in chapter VII) reveals that these are practically the same as the values calculated above for  $r$  on the assumption that no  $Na$  is complex-bound by albumin.

However there are several reasons why we consider a higher value for the Donnan ratio more probable especially in the high pH range. The first is the observation by CARR, already quoted, which suggests that at pH 7.50  $\alpha$  and  $\beta$ -globulins bind sufficient Na in a complex-type manner that the amount is not negligible at the concentrations in which these proteins are present in normal serum.

A second reason is the fact that our calculations are based on our experimental results for albumin and although the range of the individual values was similar to that found for serum, the number of experiments was considerably less. In addition to this we used the lower of the two values of corrected  $R_{Na}$ , i.e. the one calculated on the assumption that albumin depresses the flame photometer reading to such an extent that an error of 0.6 mEq/70 g albumin results. It is quite possible that the error is smaller (see chapter VII). Finally if only 1% of Na would have been complex-bound in our experiments with albumin, we calculate the Donnan ratio for serum already 0.005 higher.

If we take into consideration all available evidence, we come to the following conclusion. At pH 7.46 the value of  $r$  is probably somewhere between 0.960 and 0.965, whereas at pH 6.91 a value of about 0.970 seems the most reasonable estimate, since at the lower pH value there must be less complex bound Na respectively more complex bound Cl than at the higher pH value. We shall therefore assume that in our experiments with serum the average values were (at a protein concentration of 70 g/Kg  $H_2O$ )

$r = 0.962 \quad (\text{at pH } 7.46)$ $= 0.970 \quad (\text{at pH } 6.91)$
---

These values are somewhat arbitrary but they are not unreasonable. With some confidence it can be said that the correct value for  $r$  must have lain between 0.955 and 0.967 at pH 7.46, and between 0.965 and 0.982 at pH 6.91 (the first value of each pair was calculated on the assumption that no Na is complex bound and the second value on the assumption that not more than 1% of Cl is bound in a complex-type manner).

**The amount of ion protein-bound in a salt-type respectively complex-type manner**

We can now estimate for each ion the total amount protein-bound, as well as the fraction which is complex-bound, with the aid of equations (7) to (11) from chapter I inserting the values given for  $\Delta$  cation in chapter VII and the concentration ratios presented in table 57 and 53 (chapter VII).

$\Delta Na$  was calculated from the equation relating  $\Delta Na$  to pH given in chapter VII.  $\Delta K$  was estimated from fig. 50 in chapter VII.  $\Delta Ca + Mg$  was obtained by interpolation from table 51 (chapter VII).

The result is summarised in table 62. It can be seen that at the accepted values for  $r$  we calculate complex-bound Na to be less than 1% of the Na present in solution, even at pH 7.46. At the latter pH value complex bound Cl is calculated at 1.5% of total Cl concentration. The values calculated for K and for  $HCO_3$  are very much an approximation. The relatively large and dubious systematic error and the fairly large accidental error in the case of K, and the even larger accidental error in the case of  $HCO_3$  have already been mentioned. But table 62 suggests that for K the percentage which is com-



we calculate then from table 58 (using the lowest of the two values given for corrected  $R_{Na}$ )

complex bound Cl = 5.7 mEq/Kg  $H_2O$  at an albumin concentration of 70 g/Kg  $H_2O$

Thus we find that at pH 6.80 and at a Cl concentration of about 130 mEq/Kg  $H_2O$  5.7 moles Cl are bound per mole albumin (mol weight  $\sim 70,000$ ). This should be the maximum value since it was calculated on the assumption that no Na is complex bound. If 1 / of the Na present in the albumin solution were complex bound the Donnan ratio would be 0.964 (equation (3) in chapter I) and in that case we calculate that 4.6 moles Cl are bound per mole albumin. In a similar manner we calculate from the experiments in the high pH range (table 58) that, at pH 7.44 4.6 moles Cl are complex bound per mole albumin if no Na is complex bound and that 3.4 moles Cl must be complex-bound per mole albumin if 1 / of Na is bound in a complex-type manner.

In chapter I we came to the conclusion that the data from the literature indicate that at pH 7.40 a value between 0.5 and 3.0 is the most reasonable estimate for the moles of Cl that are complex bound per mole albumin. The values calculated above are somewhat higher - especially the one calculated on the assumption that no Na is bound in a complex type manner. In this connection it must be remembered that the results obtained by SCATCHARD *et al* by VAN OS & KOOPMAN-VAN EUPEN and by CARR do not exclude the possibility that 1-2 / of Na is complex bound at our experimental conditions.

If we now return to our main interest which is plasma under physiological conditions we cannot neglect the observation of Carr that  $\alpha$  and  $\beta$ -globulins bind Na in a complex type manner. His results appear to indicate that, at pH 7.50 2-3 mEq of Na are bound per 100 g of these proteins when the Na concentration in the solution is only 15-20 mEq/L. In the case of serum therefore we cannot consider  $R_{Na}$  to be equal to  $r$ . But since albumin is apparently the only one of the serum proteins to bind Cl in a complex type manner at physiological pH (chapter I) we can make an estimate of the Donnan ratio by first calculating the amount of complex bound Cl present in serum from the albumin concentration of the latter and from the amount of Cl bound per mole albumin as calculated from the dialysis experiments.

In our experiments 60 / of serum protein was albumin (average value found by paper electrophoresis, 60 % that found with the method of Majoor 58 %). If we assume that the relation between pH and complex-bound Cl is linear over the pH range 6.80 to 7.50, we can estimate the concentration of complex-bound Cl in serum at pH 6.91 and at pH 7.46 to be respectively 3.31 and 2.74 mEq/Kg  $H_2O$  when no Na is complex-bound by albumin, and respectively 2.67 and 2.03 mEq/Kg  $H_2O$  when 1 / of Na is complex-bound.

With the aid of equation (5) in chapter I and the data presented in table 52 (chapter VII) we then calculate the following values for the Donnan ratio of serum

	pH 6.91	pH 7.46
If no Na is complex bound by albumin	0.9666	0.9555
if 1 / of Na is complex-bound by albumin	0.9717	0.9614

A glance at the average values of  $R_{Na}$  found for normal serum (table 53 in chapter VII) reveals that these are practically the same as the values calculated above for  $r$  on the assumption that no Na is complex-bound by albumin.

From table 62 we can calculate  $\Sigma [An]_i$  by summing the concentrations found for Cl and  $HCO_3^-$ , adding 1.7 mEq for inorganic phosphate (see chapter V) and subtract the sum of complex-bound Cl,  $HCO_3^-$  and inorganic phosphate to obtain  $[An]_f$ .

c) Finally we can subtract from the value found for NCE (at 70 g/Kg  $H_2O$  protein) the difference between the sum of the quantities of Na, K, Ca and Mg and the sum of the quantities of Cl,  $HCO_3^-$  and inorganic phosphate that are complex-bound by 70 g protein. This should give us the negative net charge of the proteins (see chapter I). From the general equation for calculating NCE at a given pH value (first part of this chapter) we estimate NCE per 70 g protein to be 13.0 mEq at pH 7.46 and 8.9 mEq at pH 6.91. For the experiments on which table 52 (chapter VII) is based the average value of NCE (suitably corrected for flame photometer error and for the presence of carbamino-protein) was found to be 12.9 mEq at pH 7.46, and 8.8 mEq at pH 6.91 again per 70 g protein. In view of the fact that the general equation was derived from selected experiments and adjusted to fit both the values found with *in vivo* ultrafiltration and those found with dialysis at pH 7.35 the agreement is satisfactory. We shall use here the average values of NCE found for the experiments represented in table 52.

In table 64 the values calculated for  $[Pr^-]$  are summarised. Whether calculated according to a) or to b), identical values for  $[Pr^-]$  are found. This suggests that the equations (3), (5) and (6) in chapter I are consistent. When calculated according to c) the value of  $[Pr^-]$  is found somewhat higher at both pH values.

It must be realised that when we compare the values for  $[Pr^-]$  obtained according to a) or (b) errors in determination play no role because exactly the same set of values is inserted in the different equations. This is not so for the third method since NCE was calculated from  $([Na] + [K] + [Ca + Mg]) - ([Cl] + [HCO_3^-])$  on the assumption that the concentration of any other cation or anion present was equal on both sides of the semipermeable membrane. Na, K, Ca, Mg, Cl and  $HCO_3^-$  figure in table 52 (chapter VII) but the rest-anions do not. We could show that the assumption of equal distribution is correct for inorganic phosphate, which is the one other ion present in concentration higher than 1 mEq/L. But the assumption does not need to be true for the other anions present in serum. The non-esterified fatty acids present in normal serum in an amount of about 0.6 mEq/L are completely bound by protein. It is therefore probable that during the dialysis experiments their distribution was unequal. This may have resulted in over-estimating NCE by about 0.5 mEq per 70 g protein. Since these organic anions most increase in concentration parallel with protein, a similar error would be made in the *in vivo* ultrafiltration experiments (see chapter IV). However, the differences between the values calculated for  $[Pr^-]$  in table 64 are small and it is practically impossible to say whether they are real or the result of cumulative accidental errors. If anything they indicate that we have made slight over-estimates of NCE and not an under-estimate.

If we assume values for  $[Pr^-]$  such as 0.005 lower or higher than those on which table 64 is based, find that the absolute value of protein net charge is calculated about 1.5 mEq/70 g protein higher or lower but that the differences between the values calculated according to the three methods remain the same.

In table 65 the values obtained for the negative net charge are compared with those found for NCE. The values given for the negative net charge were calculated from the average of the values listed under b) and c) in table 61. The values given for NCE were calculated from the equation given in the first part of this chapter. It can be seen that at the lower pH value negative net charge is somewhat higher than NCE because at that pH and at the Donnan ratio indicated the total amount of complex-bound anion has become larger than the total amount of complex bound cation. From table 65 we

plex bound is probably at least as high as for Na. It suggests further that some of the  $\text{HCO}_3^-$  present in serum behaves as if complex-bound. At pH 7.46 this may be due to the fact that part of total  $\text{CO}_2$  is not  $\text{HCO}_3^-$  but carbamino-protein. The accidental error is too large to allow the relation between pH and the average value of  $R_{\text{HCO}_3^-}$  to be used for calculating the amount of carbamate present in serum. But the conclusion that some carbamino-protein probably is present at pH 7.40 is supported by the experiments with albumin and  $\gamma$ -globulin (see later).

The data on which table 62 is based offer no information concerning Ca and Mg separately. This information was obtained from the 4 dialysis experiments with plasma in which Ca + Mg as well as Ca was determined. The result is shown in table 63. Since the contribution of heparin to the non filtrable valencies must have been small (i.e. not more than 5% of the contribution of the proteins) we have assumed that the Donnan ratio was the same for plasma and for serum.

It will be seen that for Ca + Mg the values calculated are practically the same as those given in table 62, at a pH value which is nearly identical. This suggests that the values given in table 63 also apply for serum. The protein bound fraction of the cation concentration is for Mg only slightly lower than for Ca. The same holds for the percentage of protein-bound ion which is complex bound.

The percentage of inorganic phosphate which is complex-bound by 70 g normal serum protein can be estimated at 5% (see evidence presented in chapters IV and V). At the concentration used in our experiments this represents about 0.1 mEq of inorganic phosphate per 70 g protein, and this quantity has therefore been accepted in the calculation of the negative net charge of the serum proteins (see below).

In view of its key position in the calculation of the protein-bound quantity of any ion we have to consider the effect of an error in the estimation of  $r$ . If  $r$  were 0.005 lower respectively higher than the average values accepted above the calculated amount of complex bound Ca + Mg would not change significantly but for Na either no complex bound fraction would exist (when  $r$  is 0.005 lower) or it would be about 1.5 mEq/kg  $\text{H}_2\text{O}$  (when  $r$  is 0.005 higher). For Cl we would calculate the complex bound quantity 0.6 mEq/kg  $\text{H}_2\text{O}$  higher respectively the same amount lower than the quantities given in table 62.

### Negative net charge of protein

Since we are now in possession of approximative values for the amounts of complex bound cation and complex bound anion we can attempt to estimate the value of protein negative net charge ( $\text{Pr}^-$ ). This can be done in three ways.

a) We can summate the values found for protein bound Na, K, Ca and Mg (salt type and complex type), summate the values found for complex bound Na, K, Ca and Mg and subtract the latter from the first. This should give us the amount of cation balancing the negative net charge of the serum proteins.

b) If we assume the activity coefficient of the cations opposing protein charge to be the same as that of the other filtrable cations in solution we can rearrange equation (6) from chapter I to

$$[\text{Pr}^-] = \frac{[\text{An}]_i - r^2[\text{An}]_i}{r^2}$$

From table 62 we can calculate  $\Sigma [An]_i$  by summing the concentrations found for Cl and  $HCO_3^-$ , adding 1.7 mEq for inorganic phosphate (see chapter V) and subtract the sum of complex-bound Cl,  $HCO_3^-$  and inorganic phosphate to obtain  $[An]_i$ .

c) Finally we can subtract from the value found for NCE (at 70 g/Kg  $H_2O$  protein) the difference between the sum of the quantities of Na, K, Ca and Mg and the sum of the quantities of Cl,  $HCO_3^-$  and inorganic phosphate that are complex bound by 70 g protein. This should give us the negative net charge of the proteins (see chapter I). From the general equation for calculating NCE at a given pH value (first part of this chapter) we estimate NCE per 70 g protein to be 13.0 mEq at pH 7.46 and 8.9 mEq at pH 6.91. For the experiments on which table 52 (chapter VII) is based the average value of NCE (suitably corrected for flame photometer error and for the presence of carbamino-protein) was found to be 12.9 mEq at pH 7.46, and 8.8 mEq at pH 6.91 again per 70 g protein. In view of the fact that the general equation was derived from selected experiments and adjusted to fit both the values found with *in vitro* ultrafiltration and those found with dialysis at pH 7.35, the agreement is satisfactory. We shall use here the average values of NCE found for the experiments represented in table 52.

In table 64 the values calculated for  $[Pr^-]$  are summarised. Whether calculated according to a) or to b), identical values for  $[Pr^-]$  are found. This suggests that the equations (3), (5) and (6) in chapter I are consistent. When calculated according to c) the value of  $[Pr^-]$  is found somewhat higher at both pH values.

It must be realised that when we compare the values for  $[Pr^-]$  obtained according to a) or to b) errors in determination play no role because exactly the same set of values is inserted in the different equations. This is not so for the third method since NCE was calculated from  $([Na] + [K] + [Ca + Mg]) - ([Cl] + [HCO_3^-])$  on the assumption that the concentration of any other cation or anion present was equal on both sides of the semipermeable membrane. Na, K, Ca, Mg, Cl and  $HCO_3^-$  figure in table 52 (chapter VII) but the est-ions do not. We could show that the assumption of equal distribution is correct for inorganic phosphate, which is the one other ion present in concentration higher than 1 mEq/L. But the assumption does not need to be true for the other anions present in serum. The non-esterified fatty acids present in normal serum in an amount of about 0.6 mEq/L are completely bound by protein. It is therefore probable that during the dialysis experiments their distribution was unequal. This may have resulted in overestimating NCE by about 0.5 mEq per 70 g protein. Since these organic anions must increase in concentration parallel with protein, similar error would be made in the *in vivo* ultrafiltration experiments (see chapter IV). However the differences between the values calculated for  $[Pr^-]$  in table 64 are small and it is practically impossible to say whether they are real or the result of cumulative accidental errors. If anything they indicate that we have made a slight overestimate of NCE and not an underestimation.

If we assume values for  $\alpha$  which are 0.005 lower or higher than those on which table 64 is based, we find that the absolute value of protein net charge is calculated about 1.5 mEq/70 g protein higher or lower but that the differences between the values calculated according to the three methods remains the same.

In table 65 the values obtained for the negative net charge are compared with those found for NCE. The values given for the negative net charge were calculated from the average of the values listed under b) and c) in table 61. The values given for NCE were calculated from the equation given in the first part of this chapter. It can be seen that at the lower pH value negative net charge is somewhat higher than NCE because at that pH and at the Donnan ratio indicated the total amount of complex-bound anion has become larger than the total amount of complex-bound cation. From table 65 we

can derive the following rough approximation of the relation between pH and the negative net charge of the (normal) plasma proteins in the pH range 6.9 to 7.5

$$\text{negative net charge (mEq/100 g protein)} = 6.9 (\text{pH} - 4.96)$$

Finally although the *in vitro* ultrafiltration experiments do not permit direct conclusions concerning the nature of ion protein binding, we have seen in chapter IV and again in the second part of this chapter that the absence of a decrease in the sum concentration of  $\text{Cl} + \text{HCO}_3$  during haemoconcentration is a further argument for the existence of complex bound  $\text{Cl}$  (and  $\text{HCO}_3$ ?) in plasma under *in vitro* conditions

#### PATHOLOGICAL SERA AND PURIFIED PROTEIN FRACTIONS

In the previous paragraph it has become evident that we can – at best – make an estimate of the Donnan ratio in the case of normal serum. The small number of pathological sera studied makes it impossible to reach any definite conclusion as to the Donnan ratio in these cases beyond the general one that the lower the albumin concentration and the higher the (normal or pathological)  $\gamma$  globulin concentration the more the Donnan ratio will approach a value of 1.000. This conclusion is not completely correct since in the serum from one patient with myeloma the paraprotein behaved electrophoretically as a  $\gamma$ -globulin and yet the Donnan ratio must have been somewhere near 0.98 in view of the values found for  $R_N$  and  $R_{Cl}$ . But even in the sera where a value of nearly zero was found for NCE, the value found for  $R_{Ca+Mg}$  indicated that a considerable amount of the bivalent cations was bound in a complex type manner.

We have already discussed the results obtained with the purified albumin fractions as a preliminary to estimating the Donnan ratio for normal serum. A comparison with the amount of complex bound  $\text{Cl}$  estimated from experiments done by others showed a fair agreement – especially if we assume that some Na (up to 1% of total Na concentration) is complex bound also. The number of experiments done with purified  $\gamma$ -globulin was very small indeed, but the average values found for  $R_N$  and  $R_{Cl}$  were both positive, indicating that in the pH range studied the net charge of the  $\gamma$  globulins must have been positive. The difference between the two concentration ratios is definitely smaller than in the case of albumin and of normal serum, and presumably  $\text{Cl}$  is associated with  $\gamma$ -globulins in a salt type manner only.

Both in the case of albumin and of  $\gamma$ -globulin we found  $R_{\text{NCEs}}$  somewhat higher than  $R_{Cl}$  at pH 7.44 but somewhat lower at pH 6.80 (table 55 in chapter VII). The differences are smaller than those observed for normal serum, but the trend is the same. Since it is improbable that more  $\text{HCO}_3$  is complex bound at higher pH values, whereas on the other hand it is to be expected that the amount of carbamino-protein increases with increasing pH the above mentioned observation is compatible with the presence of some 0.2 mEq/Kg  $\text{H}_2\text{O}$  carbamino-protein in serum at normal pH.

*Summing up*

In this study the term binding signifies attraction between the large (non-filtrable) proteins and the small (filtrable) inorganic ions regardless of the strength and the nature of the attracting forces. For practical reasons two types of binding are assumed to exist (chapter I) the *salt*-type in which the filtrable ions are fully dissociated but statistically balance the corresponding electrostatic charge of the ionic groups on the protein molecules, and the *complex* type in which the filtrable ions merge with the protein molecules in such a manner that they no longer contribute to the ionic activity of the surrounding fluid. This is a simplification, but in a complicated solution such as plasma we cannot at present distinguish experimentally between finer differences in the nature of ion-protein binding. The distinction in salt- and complex-type of binding has the advantage that it is based on the effect of protein on the activity of the filtrable ions. In membrane equilibria it is the activity ratio which is subject to the dictates of the Donnan equilibrium and when a filtrable ion is partly protein-bound in a complex type manner the concentration ratio of that ion will differ from the Donnan ratio, the latter being equal to the activity ratio. The discrepancy can be used to calculate the complex bound fraction of the particular ion (chapter I). For the present and under the conditions existing in plasma this indirect approach appears to give values for complex-bound ion which are more accurate than those obtained from direct measurement of ion activity. But the simplifying assumptions involved should be kept in mind (chapter I).

The net effect of all protein-bound ion present in plasma manifests itself in the contribution of the proteins to the balance between cationic and anionic valencies in the solution. This contribution was termed base broding power by Van Slyke but is described more accurately by the term net cation equivalency (NCE). In chapter I the NCE of the proteins is defined as the difference between the concentrations of protein-bound filtrable cation and protein-bound filtrable anion. This includes both the ions bound in a salt-type manner and those bound in a complex-type manner. For practical reasons the negative net charge of the proteins is defined in terms of the amount of dissociated filtrable cation opposing it. NCE and negative net charge differ in magnitude when the concentrations of complex-bound cation and complex-bound anion are unequal.

In solutions such as plasma negative net charge must be calculated from NCE and the amounts of complex-bound cation respectively anion. NCE is determined indirectly by relating protein concentration to the gap which is left when the sum of filtrable anionic valencies present in plasma is subtracted from the sum of filtrable cationic valencies. The assumption is that protein is the only non-filtrable substance in plasma which binds ions.

The determination of NCE requires a high degree of accuracy in the analyses of filtrable ions and protein. Furthermore, since most proteins are more vulnerable than was previously recognised plasma should be studied in its native state or at least under conditions which imitate the *in vivo* state as closely as possible. This includes the prevention of ageing.

A study of the literature reveals (chapter II) that the value generally accepted for the NCE of the plasma proteins - 16 mEq/L plasma - is based on one publication by VAN SLYKE *et al* in 1928. Their values were obtained in experiments with albumin and globulin prepared by ammonium sulfate fractionation, prolonged dialysis and subsequent drying from two samples of horse serum and one sample of pooled human serum.

An attempt to estimate the NCE of the plasma proteins from published data on the ionic composition of normal plasma proved unrewarding mainly because of the surprisingly large spread in the values reported for the normal Na concentration and for normal total base. Furthermore the number of investigations in which Na, K, Ca, Mg as well as Cl and  $\text{HCO}_3$  were determined from the same plasma or serum samples is rather limited.

A similar conclusion was reached with regard to the published data concerning the distribution of the above mentioned ions across the capillary wall or an artificial semi permeable membrane. We could find no recent study in which the concentration ratios were determined for both cations and anions. For the Donnan ratio of the equilibrium between plasma and interstitial fluid apparently no value is available other than the one calculated from the base binding power found by Van Slyke *et al*.



In the present study we have determined the NCE of the plasma proteins under conditions closely resembling those existing *in vivo*. In addition we have made an attempt to estimate for the inorganic ions in plasma the fraction which is protein-bound in a complex type manner by determining their concentration ratios in equilibrium dialysis experiments. It was thought that these data would allow us to make a reasonable estimate of the value of the Donnan ratio for the equilibrium that normally exists between plasma and interstitial fluid.

The NCE of the plasma proteins was determined by correlating changes in protein concentration with concomitant changes in (cat min  $\text{Cl} + \text{HCO}_3$ ), i.e. the difference between the sum concentration of Na, K, Ca, and Mg and the sum concentration of Cl and  $\text{HCO}_3$ , under conditions in which the concentration of the other anions present could be assumed to be constant. To obtain samples with different protein concentrations two methods were used.

The first was *in vivo* ultrafiltration (GERBRANDY *et al* 1957). Haemoconcentration of venous forearm blood was induced by compressing the upper arm for up to 35 minutes at a pressure of about 100 mm Hg. Before, during and after compression blood samples were taken from a cubital vein. In normal subjects the maximal percentage rise in plasma protein concentration was on the average 70%. NCE was calculated by correlating the protein concentration of the samples with the values found for (cat min  $\text{Cl} + \text{HCO}_3$ ) and the concentration of the rest anions (RA) was obtained by extra

polating to protein concentration zero. In a similar manner values were obtained for protein-bound and 'free' cation.

The second method used was equilibrium dialysis. Samples of fresh serum or plasma were dialysed at 37.5°C against a synthetic interstitial fluid using a cellulose membrane. The pH value was varied by changing both  $\text{HCO}_3^-$  concentration and  $\text{Pco}_2$ . NCE was calculated by correlating the difference between the values found for (cat min Cl +  $\text{HCO}_3^-$ ) inside and outside the membrane with the protein concentration inside. This was also done for the individual cations in order to obtain data concerning protein-bound cation. From the same experiments concentration ratios were calculated for Na, K, Ca + Mg, Cl and  $\text{HCO}_3^-$ .

Both NCE and protein-bound cation were expressed in mEq per 100 g protein in order to eliminate the effect of differences in protein concentration. For similar reasons all concentration ratios were calculated for a protein concentration of 70 g/Kg  $\text{H}_2\text{O}$  which is about the average of the protein concentrations observed in the dialysis or ultrafiltration experiments reported by others.

Quite a number of assumptions are involved in calculating NCE and protein-bound cation in the manner described. Moreover the summation of analytical data may seriously affect the accuracy of the values obtained especially in the case of NCE. Chapter III is therefore devoted to the discussion of the systematic and accidental errors of the methods used for chemical analysis. In this respect the macro-Kjeldahl, the buret method and the flame photometer analysis must be mentioned in particular. The latter technique was found to give a rather large positive error for Na when deproteinised plasma is analysed but only a small negative error when plasma is analysed. The evidence presented in chapter III suggests that chemical analysis was sufficiently accurate to permit the determination of NCE in the manner explained above.

In chapter IV the technique of *in vivo* ultrafiltration is described together with the results of experiments devised to detect errors in the use of this method for the determination of NCE. It was found that - provided the arm is kept completely relaxed throughout the course of the experiment - the physico-chemical changes resulting from venous stasis during 45 minutes do not seriously affect the calculation of NCE in the manner described. The relationship between protein concentration and (cat min Cl +  $\text{HCO}_3^-$ ) was found to be rectilinear over the protein range encountered in our experiments; the protein composition remained unaltered during haemoconcentration; no significant change was observed in inorganic phosphate or lactate content (at least not in subjects without anaemia); the change in pH remained sufficiently small to allow a correction to be made for its effect on NCE. In chapter V the technique of equilibrium dialysis is discussed and it appears that reasonably accurate values can be obtained for NCE and for the concentration ratios of the various ions studied. An exception must be made for the H-ions: the pH of the samples taken from the cellulose bags tended to be higher than the pH of the dialysis solution surrounding them. The difference was small but sufficient to prevent the use of the H-ion concentration ratio as an indicator of the Donnan ratio.

With regard to the determination of protein-bound cation it was concluded that in the case of Ca and Mg the systematic errors remain relatively small with both methods.



In the case of Na and K however the Donnan equilibrium is responsible for a large systematic error which can only be corrected for if the Donnan ratio is known. Even then the values obtained for protein-bound K remain unreliable in the case of *in vitro* ultrafiltration because of unpredictable changes in the concentration of free K.

In chapter VI the results obtained with *in vitro* ultrafiltration are presented and the magnitude of the systematic errors is discussed. 28 healthy men (22 to 42 yrs old) and 29 patients (both male and female, aged 25 to 87 yrs) were studied in the fasting state. The patients were selected because of an abnormal composition of the plasma proteins or an abnormality of the plasma ions.

Chapter VII presents the results obtained by means of equilibrium dialysis at pH values ranging from 6.3 to 8.0. Serum in a few instances plasma, from 19 normal subjects (1 female) was dialysed. 15 of whom had also been studied by *in vitro* ultrafiltration. Only four pathological sera were dialysed. They were obtained from patients with myeloma and 1 patient with macroglobulinaemia. In addition a number of experiments were done with albumin and with  $\gamma$  globulin solutions.

In chapter VIII the results obtained are compared and conclusions are drawn concerning the Donnan ratio and the amounts of ion bound in a complex-type manner. With either method nearly the same average value was found for the NCE of the normal plasma or serum proteins at pH 7.35. Further evidence that the two methods give comparable results is found in the observation that the dialysis experiments with serum from patients with myeloma gave similar results as *in vitro* ultrafiltration in patients with the same disease. The results obtained for protein bound cation were also similar when the systematic error resulting from the Donnan equilibrium was taken into account. Finally although the experiments were done in the course of one and a half year we could detect no seasonal changes (chapter VI and Addendum). The results obtained with the two methods have therefore been averaged and possible differences between true plasma and serum neglected.

★

Our results can be summarised as follows (the values given are those found after correction for systematic errors).

For the normal subjects the average value of NCE was 18 mEq/100 g protein at pH 7.40. The individual values were within 4 mEq of the average. When expressed per litre plasma the normal range for NCE was found to be 10-15 mEq/L with an average value of 12.5 mEq/L. In all normal subjects NCE was lower than 24 mEq/100 g protein the value to be expected from the equation given by Van Slyke *et al* in 1928 and which can be written as

$$\text{NCE (mEq/100 g protein)} = 10.4 (\text{pH} - 5.08).$$

The difference was observed over the whole of the pH range studied and consequently we found the same absolute change in NCE per unit pH change as did Van Slyke and coworkers. This is born out by the equation which describes our results in the pH range

6.60-7.70 for plasma or serum with a normal protein composition and normal concentrations for the inorganic ions

$$\text{NCE (mEq/100 g protein)} = 10.3 (\text{pH} - 5.66).$$

Van Slyke *et al.* and others with them, have tacitly assumed 'base binding power' to be identical with the negative net charge of the proteins. This is erroneous (chapter I) but in the case of the plasma proteins the quantitative difference is not large at least not in the pH range observed in medicine. For reasons discussed in chapter VIII we estimate that at pH 7.40 the absolute value of the negative net charge of the plasma proteins is about  $\frac{1}{2}$  lower than NCE. The discrepancy increases with increasing pH but becomes smaller at lower pH values and at pH 6.90 the negative net charge is even greater than NCE (about  $\frac{5}{2}$ ). A rough approximation of the correlation between pH and negative net charge of the normal plasma proteins in the pH range 6.9 to 7.5 is given by the equation

$$\text{negative net charge (mEq/100 g protein)} = 6.9 (\text{pH} - 4.96).$$

The distinction between NCE and negative net charge is not an academic one since only the latter should be used in the equations describing the Donnan equilibrium. It must however be stressed that the values given for negative net charge have been estimated on the assumption that ion-protein interaction is either of the salt-type or of the complex-type. Therefore they do not necessarily correspond with the exact number of negatively charged univalent ionic groups on the proteins present in excess of positively charged groups (chapter I).

The above estimate of negative net charge was calculated on the assumption that in our dialysis experiments the Donnan ratio for the equilibrium between normal serum and dialysis solution was on the average 0.962 at pH 7.46 and 0.970 at pH 6.91 (protein concentration 70 g/Kg  $\text{H}_2\text{O}$ ). These values were not determined directly but estimated from circumstantial evidence including besides our own experiments observations made by Scatchard *et al.* and by Carr on purified albumin (chapter VIII). The fact that during the *in vivo* ultrafiltration experiments the concentration of  $\text{Cl} + \text{HCO}_3$  remained constant suggests that in normal subjects the Donnan ratio for the equilibrium existing across the capillary wall is of the order of 0.97 (chapter IV). These values are decidedly higher than those previously estimated for the Donnan ratio (MANERY 1954). But it should be stressed that values reported for the Donnan ratio and for the concentration ratios of the different ions can only be compared correctly by taking into account differences in pH, in protein concentration, in protein composition and in the concentration of the filtrable ions.

With regard to the concentration ratios of the inorganic ions our results confirm the conclusion of Manery that there is a significant difference between the values obtained for the univalent cations on the one hand and those obtained for the univalent anions on the other. This suggests that part of the univalent ions is bound in a complex-type manner by normal plasma proteins.

In the case of Na and Cl our results appear to be fairly accurate and comparison of the relationship between their respective concentration ratios and the pH strongly suggests that at pH 6.90 the plasma proteins bind a significant amount of Cl in a complex

type manner. By taking into account the results obtained by Scatchard *et al.*, by Carr and by ourselves with albumin solutions we could estimate the amount of complex bound Cl to be about 2 mEq/L in normal plasma at a pH value of 7.40. From the experiments reported by Carr one must conclude that this Cl is attached to albumin. Evidence presented by other workers and our own observations suggest that under physiological conditions the amount of Na which is protein bound in a complex type manner is at the highest 1.0 to 1.5 mEq/L plasma. This is about 10% of the total amount of protein-bound Na in normal plasma which we calculated at 12 mEq/L. Again evidence presented by Carr suggests that it are mainly  $\alpha$  and  $\beta$ -globulin and fibrinogen which are responsible for the complex type binding of Na.

For K and  $\text{HCO}_3$  our results are much more equivocal because of the relatively large accidental – and in the case of K systematic – errors. When corrected for the latter the concentration ratio of K is on the average somewhat lower than the ratio found for Na suggesting that relatively more K is bound in a complex type manner. In the case of  $\text{HCO}_3$  the concentration ratio tends to be higher than the Cl ratio at pH 7.40 but lower at pH 6.90. This observation is compatible with the presence of a small amount (about 0.2 mEq/L plasma) of carbamino-protein at the higher pH value.

As our main interest was in the univalent ions, Ca and Mg were determined separately in only 4 of the dialysis experiments. The results, together with those obtained by means of *in vivo* ultrafiltration indicate that for Mg the protein bound fraction is relatively smaller than for Ca, at least in normal plasma. It is interesting to note that CARR (1955) and VAN OS & KOOPMAN-VAN EUPEN (1957) – both measuring complex bound ion – found no difference in the affinity of albumin for Ca and Mg whereas IRONS & PERKINS (1962) – who studied among other things the effect of albumin on the distribution of radioactive Ca and Mg isotopes in equilibrium dialysis – concluded that the affinity for Ca was decidedly higher than that for Mg. From the 4 experiments in which we determined the concentration ratio both for Ca and for Mg it would appear that in plasma the percentage of the total amount of protein bound ion which is complex bound is nearly the same for Ca and Mg (about 75%). The correlation between total protein bound ion (in mEq/100 g protein) and pH was only studied for the sum of Ca and Mg. It was distinctly curvilinear, the protein bound fraction increasing progressively with increasing pH (chapter VII). LOKEN *et al* (1960) in their ultracentrifugal analysis of protein bound and 'free' Ca in human serum observed a similar relationship in the pH range 6.5–7.5 but in their case the correlation was practically linear from pH 7.0 upwards. In the ultrafiltration experiments reported by TORIBARA *et al* (1957) and HOPKINS *et al* (1952) the correlation between protein bound Ca and pH appeared to be linear over the pH range 6.0–8.0 but the number of observations in which the pH value was lower than 7.4 was very small.

We have expressed our normal values per unit weight of plasma protein with the restriction that the composition of the latter be normal. This addition is necessary since the relative contribution of the various protein fractions to NCE etc. differs. VAN SLYKE *et al* in 1928 did not determine the NCE of serum but separated the serum proteins in an albumin and a globulin fraction, determined NCE for each fraction and then calculated the NCE to be expected for total serum protein. At pH 7.40 they found NCE to be 28 mEq/100 g albumin and 19 mEq/100 g globulin. We use quotation marks to

stress the fact that the fractions studied by Van Slyke differ from the fractions isolated by means of electrophoresis. We have studied electrophoretically pure albumin and  $\gamma$ -globulin but no other fractions, and we can therefore not predict the NCE of total plasma protein. But the values which we obtained at pH 7.40 for the NCE of albumin (20 to 22 mEq/100 g) and that of  $\gamma$ -globulin ( $-2$  to 0 mEq/100 g) differ considerably from those found by Van Slyke *et al* for their albumin and globulin fractions. In fact if we consider the amounts of albumin and  $\gamma$ -globulin normally present in plasma and compare the NCE found for albumin and for  $\gamma$ -globulin with the NCE found for plasma protein we must conclude either that the NCE of the protein fractions studied by us has decreased during the process of fractionation or that the  $\alpha$ - and/or the  $\beta$ -globulins have a much higher NCE than albumin. The normal value found for NCE in patients with a relatively high  $\alpha$ - and  $\beta$ -globulin but a low albumin content of their plasma does agree with this conclusion (chapter VI). It is also in keeping with the observation of Carr that the  $\alpha$ - and  $\beta$ -globulins bind Na (and K) in a complex-type manner.

It must be concluded that an accurate prediction of the NCE of plasma protein from its (electrophoretic) composition is not possible. Roughly speaking the higher the albumin or the  $\alpha$ - and  $\beta$ -globulin content of the plasma the higher NCE, the higher the  $\gamma$ -globulin content the lower NCE will be. But even this approximative estimate may lead one astray since proteins which in routine paper electrophoresis behave as  $\gamma$ -globulins apparently can have a NCE which is about equal to that of albumin as we observed in one patient with myeloma (chapter VII).

The behaviour of the different ions in the presence of albumin and of  $\gamma$ -globulin leads to the following conclusions. Whereas at comparable pH values the difference between the concentration ratios was larger in the case of albumin than in the case of serum, no significant difference was observed in the experiments with  $\gamma$ -globulin. But in the latter case both ratios were at pH 7.40 higher than 1.000, which indicates that at that pH value the net charge of  $\gamma$ -globulin is positive. At the same pH however  $\gamma$ -globulin binds Ca and Mg in significant amounts - obviously in a complex-type manner. The observation lends support to the opinion that part of the Ca and Mg binding by plasma proteins does not depend on electrostatic attraction.

In the case of the patients the results obtained by means of *in vitro* ultrafiltration were less accurate because haemoconcentration was generally less pronounced and the number of blood samples smaller than in the experiments with normal subjects. Even so NCE and protein-bound cation expressed per 100 g protein, were within the normal range for nearly all patients including those with severe uraemia and the 2 patients with a  $\beta$ -type of myeloma. The exception were the 3 patients with a  $\gamma$ -type of myeloma for whom both NCE and protein-bound cation were found to be considerably lower than normal. The one patient with a  $\gamma$ -type of myeloma and yet a normal value for NCE has already been mentioned. When the actual values of NCE per litre plasma are compared the conclusion is different because of the abnormally high or low plasma protein concentration symptomatic for certain disease states. In one patient with a  $\gamma$ -type of myeloma and a total protein concentration of 149 g/L plasma we found NCE 12 mEq/L plasma (which is normal) but 8 mEq/100 g protein (which is definitely too low). The reverse was observed in patients with hypoproteinaemia. But, whether expressed per unit volume of plasma or per unit weight of protein NCE was always found to have a positive value.

type manner. By taking into account the results obtained by Scatchard *et al* by Carr and by ourselves with albumin solutions we could estimate the amount of complex bound Cl to be about 2 mEq/L in normal plasma at a pH value of 7.40. From the experiments reported by Carr one must conclude that this Cl is attached to albumin. Evidence presented by other workers and our own observations suggest that under physiological conditions the amount of Na which is protein bound in a complex type manner is at the highest 1.0 to 1.5 mEq/L plasma. This is about 10% of the total amount of protein bound Na in normal plasma which we calculated at 12 mEq/L. Again evidence presented by Carr suggests that it are mainly  $\alpha$  and  $\beta$ -globulin and fibrinogen which are responsible for the complex type binding of Na.

For K and  $\text{HCO}_3$  our results are much more equivocal because of the relatively large accidental – and in the case of K systematic – errors. When corrected for the latter the concentration ratio of K is on the average somewhat lower than the ratio found for Na suggesting that relatively more K is bound in a complex type manner. In the case of  $\text{HCO}_3$  the concentration ratio tends to be higher than the Cl ratio at pH 7.40 but lower at pH 6.90. This observation is compatible with the presence of a small amount (about 0.2 mEq/L plasma) of carbamino-protein at the higher pH value.

As our main interest was in the univalent ions Ca and Mg were determined separately in only 4 of the dialysis experiments. The results, together with those obtained by means of *in vitro* ultrafiltration indicate that for Mg the protein bound fraction is relatively smaller than for Ca, at least in normal plasma. It is interesting to note that CARR (1955) and VAN OS & KOOPMAN VAN EUPEN (1957) – both measuring complex-bound ion – found no difference in the affinity of albumin for Ca and Mg whereas IRONS & PERKINS (1962) – who studied among other things the effect of albumin on the distribution of radioactive Ca and Mg isotopes in equilibrium dialysis – concluded that the affinity for Ca was decidedly higher than that for Mg. From the 4 experiments in which we determined the concentration ratio both for Ca and for Mg it would appear that in plasma the percentage of the total amount of protein bound ion which is complex bound is nearly the same for Ca and Mg (about 75%). The correlation between total protein bound ion (in mEq/100 g protein) and pH was only studied for the sum of Ca and Mg. It was distinctly curvilinear the protein bound fraction increasing progressively with increasing pH (chapter VII). LOREN *et al* (1960) in their ultracentrifugal analysis of protein bound and free Ca in human serum observed a similar relationship in the pH range 5–7.5 but in their case the correlation was practically linear from pH 7.0 upwards. In the ultrafiltration experiments reported by TORIBARA *et al* (1957) and HOPKINS *et al* (1952) the correlation between protein bound Ca and pH appeared to be linear over the pH range 5.0–8.0 but the number of observations in which the pH value was lower than 7.4 was very small.

We have expressed our normal values per unit weight of plasma protein with the restriction that the composition of the latter be normal. This addition is necessary since the relative contribution of the various protein fractions to NCE etc. differs. VAN SLYKE *et al* in 1928 did not determine the NCE of serum but separated the serum proteins in an albumin and  $\beta$  globulin fraction determined NCE for each fraction and then calculated the NCE to be expected for total serum protein. At pH 7.40 they found NCE to be 28 mEq/100 g albumin and 19 mEq/100 g globulin. We use quotation marks to

stress the fact that the fractions studied by Van Slyke differ from the fractions isolated by means of electrophoresis. We have studied electrophoretically pure albumin and  $\gamma$ -globulin but no other fractions, and we can therefore not predict the NCE of total plasma protein. But the values which we obtained at pH 7.40 for the NCE of albumin (20 to 22 mEq/100 g) and that of  $\gamma$ -globulin ( $-2$  to 0 mEq/100 g) differ considerably from those found by Van Slyke *et al* for their albumin and globulin fractions. In fact if we consider the amounts of albumin and  $\gamma$ -globulin normally present in plasma and compare the NCE found for albumin and for  $\gamma$ -globulin with the NCE found for plasma protein, we must conclude either that the NCE of the protein fractions studied by us has decreased during the process of fractionation or that the  $\alpha$  and/or the  $\beta$ -globulins have a much higher NCE than albumin. The normal value found for NCE in patients with a relatively high  $\alpha$  and  $\beta$ -globulin but a low albumin content of their plasma does agree with this conclusion (chapter VI). It is also in keeping with the observation of Carr that the  $\alpha$  and  $\gamma$ -globulins bind Na (and K) in a complex type manner.

It must be concluded that an accurate prediction of the NCE of plasma protein from its (electrophoretic) composition is not possible. Roughly speaking the higher the albumin or the  $\alpha$  and  $\beta$ -globulin content of the plasma the higher NCE, the higher the  $\gamma$ -globulin content the lower NCE will be. But even this approximate estimate may lead one astray since proteins which in routine paper electrophoresis behave as  $\gamma$ -globulins apparently can have a NCE which is about equal to that of albumin as we observed in one patient with myeloma (chapter VII).

The behaviour of the different ions in the presence of albumin and of  $\gamma$ -globulin leads to the following conclusions. Whereas at comparable pH values the difference between the concentration ratios was larger in the case of albumin than in the case of serum, no significant difference was observed in the experiments with  $\gamma$ -globulin. But in the latter case both ratios were at pH 7.40 higher than 1.000, which indicates that at that pH value the net charge of  $\gamma$ -globulin is positive. At the same pH, however  $\gamma$ -globulin binds Ca and Mg in significant amounts - obviously in a complex-type manner. The observation lends support to the opinion that part of the Ca and Mg binding by plasma proteins does not depend on electrostatic attraction.

In the case of the patients the results obtained by means of *in vivo* ultrafiltration were less accurate because haemoconcentration was generally less pronounced and the number of blood samples smaller than in the experiments with normal subjects. Even so NCE and protein-bound cation expressed per 100 g protein, were within the normal range for nearly all patients including those with severe uraemia and the 2 patients with a  $\mu$ -type of myeloma. The exception were the 5 patients with a  $\gamma$ -type of myeloma for whom both NCE and protein-bound cation were found to be considerably lower than normal, the one patient with a  $\gamma$ -type of myeloma and yet a normal value for NCE has already been mentioned. When the actual values of NCE per litre plasma are compared the conclusion is different because of the abnormally high or low plasma protein concentration symptomatic for certain disease states. In one patient with a  $\gamma$  type of myeloma and a total protein concentration of 149 g/L plasma we found NCE 12 mEq/L plasma (which is normal) but 8 mEq/100 g protein (which is definitely too low). The reverse was observed in patients with hypoproteinaemia. But, whether expressed per unit volume of plasma or per unit weight of protein, NCE was always found to have a positive value.

in the physiological pH range even in the case of serum from patients with a  $\gamma$  type of myeloma the pH had to be lowered to 6.90 before the value of NCE decreased to zero. We are therefore inclined to think that the transiently negative values for NCE observed by Broch in patients with fairly normal plasma pH and protein composition were due to errors in determination.

The most intriguing of our results is the low value found for NCE in normal subjects. The difference with Van Slyke's value is too large to be explained by accidental errors and the evidence presented in previous chapters makes it unlikely that it is due to systematic errors in our determinations. It is possible that in Van Slyke's experiments the fractionation and subsequent treatment of the proteins have increased their NCE by removing adhering substances such as fatty acids. Furthermore some of our observations suggest that prolonged storage of plasma or serum tends to increase the NCE of the proteins (chapter V). Still another explanation is suggested by the fact that during dialysis of the isolated protein fractions Van Slyke *et al.* kept the pH of the solutions at about 5.0 so as to remove all traces of cation other than H<sup>+</sup> ion. Since it is now known that at pH values below 6.0 albumin binds a considerable amount of Cl in a complex type manner it is conceivable that whereas all Na had been removed the albumin used in the experiments of Van Slyke *et al.* still contained some Cl. In the final titration Cl concentration was not determined but calculated on the assumption that the weighed amount of NaCl added to the dialysed protein solutions accounted for all of the Cl present. As the base binding power of the proteins was obtained by subtracting from the Na concentration the concentration of Cl + HCO<sub>3</sub>, neglect of an extra quantity of Cl added with the albumin would lead to calculating erroneously high values for NCE. The high value found for globulin can partly be explained by the fact that the preparation studied by Van Slyke *et al.* must have contained  $\alpha$  and  $\beta$ -globulin besides  $\gamma$  globulin. Another contribution to the calculation of erroneously high values for NCE may have come from a systematic negative error in the determination of protein concentration. Van Slyke *et al.* used a factor of 6.25 for converting nitrogen concentration into protein concentration but later studies by Van Slyke and co-workers (HILLER *et al.* 1948) suggest that with the Kjeldahl method used in 1928 a nitrogen factor of 6.5 would have been more correct. The resulting error would account for one fifth of the difference between the NCE value found by Van Slyke *et al.* and the value found by us. Although several possibilities suggest themselves we must conclude that we have no definite explanation for the difference observed.



We can use our results to draw a picture of the ionic composition of normal plasma. Table 66 shows our values for normal plasma (venous blood from the resting forearm). For the filtrable ions the values were taken from table 40 but NCE was calculated from the equation describing the correlation between pH and NCE. As a result the value of RA is 0.4 mEq/Kg H<sub>2</sub>O higher than in table 42; the difference is acceptable since even for the non-corrected values of RA 2 SE of the mean is larger than that (Addendum). Table 66 should be compared with table 2 in which the results obtained by others are given. Strictly speaking our values only apply to fasting men aged 20 to 40 yrs and they

TABLE 66 - Average values for normal venous plasma (fasting state)

pH		7.39	
protein concentration		68.4 g/L (72.8 g/Kg H <sub>2</sub> O)	
protein composition			
albumin		60%	
α <sub>1</sub> -globulin		4%	
α <sub>2</sub> -globulin		8%	
β-globulin		7%	
fibrinogen		6%	
γ-globulin		15%	
cations		anions	
Na	139.0 (147.9)	Cl	106.0 (112.8)
K	4.0 (4.3)	HCO <sub>3</sub>	26.1 (27.8)
		RA	5.1 (5.4)
Ca	4.7 (5.0)	inorg phosph	1.9
		lactate	1.4
Mg	1.7 (1.8)	NCE	12.2 (13.0)
sum	149.4 (159.0)	sum	149.4 (159.0)

The first value indicates the concentration in mEq/L plasma, the value between brackets the concentration in mEq/Kg H<sub>2</sub>O.

may be somewhat different in the very young and the very old. We found the Na concentration somewhat lower and the Cl concentration somewhat higher than the average of the values quoted in table 2. Indeed our value for the normal Cl concentration is definitely higher; however we studied fasting subjects and the observation by FAWCETT & WYNN (1956) that after a meal the plasma Cl concentration tends to decrease may explain the fact that our values are relatively high. Inorganic phosphate and lactate concentration were determined in part of the normal subjects only but when these values are subtracted from the 5.0 mEq/Kg H<sub>2</sub>O actually calculated for RA from our *in vivo* ultrafiltration experiments we find that 1.5 mEq/L are left for SO<sub>4</sub> and the remaining organic acids. In view of the data quoted in table 3 this is not unreasonable. The pH value given in table 66 needs some comment. The average of the pH values actually measured was 7.36. But pH was determined at a temperature of 37.5°C and evidence presented in chapter IV strongly suggests that the temperature of the blood in the cubital veins is about 2°C lower. The average pH value was therefore corrected by adding 0.03.

Table 67 gives the Donnan ratio and the concentration ratios for the equilibrium between normal plasma and artificial interstitial fluid at a pH value of 7.40 and a temperature of 37.5°C. It was constructed from the evidence presented in chapters VII and VIII and should be compared with table 4. We found the ratio for Na considerably higher than the values previously reported, whereas no such difference was found for Cl. In view of the small spread in our individual values and in view of the special efforts to avoid systematic errors we believe our values to be more accurate than those given in table 4. In reality the distribution across the capillary wall will be slightly different



in the physiological pH range even in the case of serum from patients with a  $\gamma$  type of myeloma the pH had to be lowered to 6.90 before the value of NCE decreased to zero. We are therefore inclined to think that the transiently negative values for NCE observed by Broch in patients with fairly normal plasma pH and protein composition were due to errors in determination.

The most intriguing of our results is the low value found for NCE in normal subjects. The difference with Van Slyke's value is too large to be explained by accidental errors and the evidence presented in previous chapters makes it unlikely that it is due to systematic errors in our determinations. It is possible that in Van Slyke's experiments the fractionation and subsequent treatment of the proteins have increased their NCE by removing adhering substances such as fatty acids. Furthermore some of our observations suggest that prolonged storage of plasma or serum tends to increase the NCE of the proteins (chapter V). Still another explanation is suggested by the fact that during dialysis of the isolated protein fractions Van Slyke *et al.* kept the pH of the solutions at about 5.0 so as to remove all traces of cation other than H<sup>+</sup> ion. Since it is now known that at pH values below 6.0 albumin binds a considerable amount of Cl in a complex type manner it is conceivable that whereas all Na had been removed the albumin used in the experiments of Van Slyke *et al.* still contained some Cl. In the final titration Cl concentration was not determined but calculated on the assumption that the weighed amount of NaCl added to the dialysed protein solutions accounted for all of the Cl present. As the base binding power of the proteins was obtained by subtracting from the Na concentration the concentration of Cl + HCO<sub>3</sub>, neglect of an extra quantity of Cl added with the albumin would lead to calculating erroneously high values for NCE. The high value found for globulin can partly be explained by the fact that the preparation studied by Van Slyke *et al.* must have contained  $\alpha$  and  $\beta$ -globulin besides  $\gamma$  globulin. Another contribution to the calculation of erroneously high values for NCE may have come from a systematic negative error in the determination of protein concentration. Van Slyke *et al.* used a factor of 6.25 for converting nitrogen concentration into protein concentration but later studies by Van Slyke and co-workers (HILLER *et al.* 1948) suggest that with the Kjeldahl method used in 1928 a nitrogen factor of 6.5 would have been more correct. The resulting error would account for one fifth of the difference between the NCE value found by Van Slyke *et al.* and the value found by us. Although several possibilities suggest themselves we must conclude that we have no definite explanation for the difference observed.



We can use our results to draw a picture of the ionic composition of normal plasma. Table 66 shows our values for normal plasma (venous blood from the resting forearm). For the filtrable ions the values were taken from table 40, but NCE was calculated from the equation describing the correlation between pH and NCE. As a result the value of RA is 0.4 mEq/Kg H<sub>2</sub>O higher than in table 42; the difference is acceptable since even for the non-corrected values of RA 2 SE of the mean is larger than that (Addendum). Table 66 should be compared with table 2 in which the results obtained by others are given. Strictly speaking our values only apply to fasting men aged 20 to 40 yrs and they

In fig. 55 we have attempted to construct a cation-anion diagram for normal plasma after the manner of Gramble, allowing for the presence of complex-bound ions in the quantities estimated in chapter VIII. The latter are placed below the level of the gross horizontal line. Fig. 55 can be compared with D in fig. 7 (chapter I) by turning the latter upside down. Fig. 55 should be a reasonably accurate representation of the actual situation existing in plasma. However when systematic errors are made in one or more of the analyses of the individual ions the values given above for NCE and RA will not fit. This is demonstrated by the values obtained in the experiments in which flame photometer analysis was done on deproteinised plasma instead of plasma (chapter VI). This stresses once again the fact that we possess no direct determination of NCE. As a consequence all values reported, including ours, must be viewed with the disturbing effect of cumulative systematic errors in mind.

Our observations suggest that *in vivo* ultrafiltration can indeed be used for the determination of NCE in an individual patient with an accuracy of plus or minus 4 mEq/L plasma. Whether this will be a help in solving clinical problems remains to be seen but the finding of an unexpectedly high value for NCE in a patient with a  $\gamma$  type of myeloma suggests that also in this respect there is more to the plasma proteins than meets the eye at present. This is also true for the binding of the individual ions as demonstrated by the interesting observation of abnormal Ca binding reported by GLUZICK *et al* (1962). A distinct disadvantage of the method of *in vivo* ultrafiltration is the loss of blood involved which prevents at present frequent determinations in the same subject. The use of micro-methods for chemical analysis would considerably reduce the amount of plasma needed but the accuracy necessary is such that the introduction of a new analytical method necessitates a thorough search for systematic errors. The experience of others and of ourselves with the flame photometer analysis of plasma Na and K illustrates that it can be very difficult to exclude systematic errors with certainty. The problem of systematic errors also looms large in the determination of the amount of complex bound ion in plasma. We could only make an estimate by using circumstantial evidence. It will be interesting to see whether the specialised electrodes now coming into use can be made sufficiently selective to measure ion activity in plasma without systematic error. The problems involved are illustrated by the fact that it is still an open question whether the present glass-electrodes really measure H-ion activity.

Finally the question remains whether the NCE of the plasma proteins serves a biological purpose and has a regulatory function in the maintenance of the ionic composition of extracellular fluid. Contrary to Broch we find this rather difficult to believe. Whereas changes in the concentration of special protein fractions may conceivably contribute to the control of the level of free Ca or Mg, the NCE of the plasma proteins is the resultant of so many different protein-ion interactions that we fail to see how NCE per se can be part of a regulating mechanism even as a buffer substance for H-ion concentration the plasma proteins are ineffective. Rather NCE should be considered a by-product of the fact that proteins are ionic substances. This does not mean that the determination of NCE is a waste of energy. The observation that it may differ considerably from the value expected from electrophoretic protein composition suggests that NCE is one of the characteristics by which plasma proteins can be differentiated. But

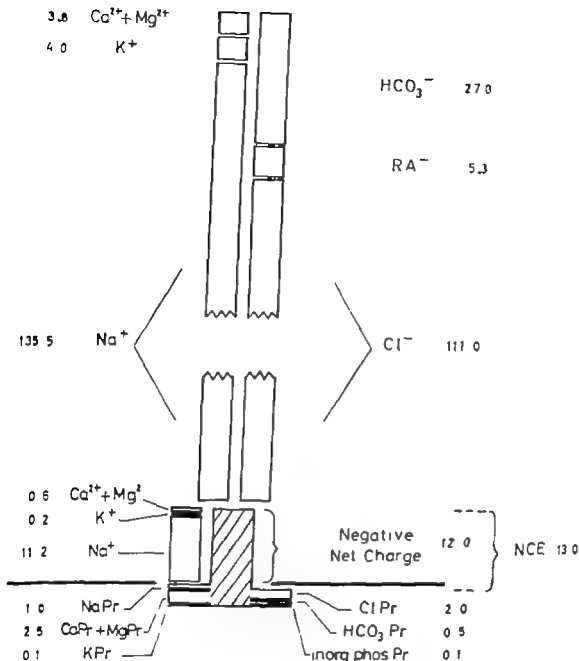


Fig. 55 Ionic composition of normal plasma (all values in mEq/kg  $\text{H}_2\text{O}$ )

as a result of the presence of non filtrable ionic substances in the interstitial fluid. The latter will increase all ratios but at present it is impossible to say how much

TABLE 67 — Donnan ratio and concentration ratios for the equilibrium between plasma and artificial interstitial fluid at pH 7.40 and temperature 37.5 °C plasma protein concentration 70 g/Kg  $\text{H}_2\text{O}$  protein composition normal

r	$R_{\text{Ca}}$	$R_{\text{Mg}}$	$R_{\text{K}}$	$R_{\text{Na}}$	$R_{\text{Cl}}$	$R_{\text{HCO}_3}$
0.963	0.57	0.65	0.94	0.956	0.979	0.99

## List of Bibliographical References

- ALBERT, R. A. & H. H. MARVIN (1931) *J. Amer. chem. Soc.* 53: 3220.
- ALDRIDGE, H. & C. KORTH (1932) *Deutsche Arch. klin. Med.* 177: 412.
- ANDERSON, R., O. CADDE & K. L. ZIEGLER (1954) *J. clin. Invest.* 33: 671.
- ANNAS, J. S. & A. S. REILMAN (1939) *Amer. J. clin. Path.* 31: 155.
- ARTZ, P. (1954) *Scand. J. clin. Lab. Invest.* 8: 33.
- ATCHELY, D. W., R. F. LOCK, E. M. REDFORD & W. M. PALMA (1923) *Arch. intern. Med.* 31: 611.
- ATCHELY, D. W. & E. M. REDFORD (1930) *J. clin. Invest.* 9: 265.
- ADWITZ, H. (1974) *Veröffentlichungen für Dermatologie und Syphilis* 1: 375.
- BALZAN, M. A. R., ANDERSON, G., CADDE & K. L. ZIEGLER (1952) *J. clin. Invest.* 31: 116.
- BALDER, E. B. & W. H. SUMMATION (1941) *J. biol. Chem.* 138: 533.
- BATES, R. G. (1954) *Electrocardiography: Principles, Theory and Practice*. New York.
- BROCKEN, W. M. & J. SHAPPEL (1933) *Biochem. Z.* 260: 105.
- BROCKHOFF, H. (1932) *Ergebn. inn. Med. und Kinderheilkunde*, 42: 273.
- BRODMAN, L. B., BALZAN & B. SCHULZ (1963) *Lancet* 1: 992.
- BROCKTHORN, J. (1962) *Scand. J. clin. Lab. Invest.* 14: Suppl. 66.
- BROCKTHORN, J. & E. HULTHORN (1962) *Lancet* 2: 132.
- BURNETT, R. E. (1952) *S. Afr. J. med. Sci.* 17: 101.
- BUTL, A. (1908) *Hygiene als Hindernis*, 4th ed. Leipzig.
- BUTL, F. W. (1945) *Amer. J. med. Sci.* 210: 195.
- BLACKMAN, K. D. & B. HAMILTON (1927) *Brit. Hopkin. Hosp.* 1: 322.
- BLOTT, H. L. (1954) *Lehrbuch der physikalischen Biochemie*. Harlow & London.
- BOYS, A. A. (1942) *De bepaling van de myofibrillen en het mitochondriaal area sediment onderzoek bij subcutaan injectie van parathyroïdine. Thesis, A. cum.*
- BOYCE, O. J. (1945) *Acta med. Scand. Suppl.* 166.
- BOYCE, O. J. (1953) *Scand. J. clin. Lab. Invest.* 5: 9.
- BOYCE-BOYCE DE BOYCE, H. G. G. and associates (1949) in KRUTY, H. R. (ed). *Colloid Science vol 11*. Amsterdam.
- CARR, CH. W. (1953) *Arch. Biochem.* 43: 167.
- CARR, CH. W. (1953) *Arch. Biochem.* 45: 417.
- CARR, CH. W. (1953) *Proc. Soc. exp. Biol. Med.* (N. Y.) 89: 346.
- CARR, CH. W. (1956) *Arch. Biochem.* 62: 476.
- CHILL, H. J. & A. B. HASTINGS (1938) *J. clin. Invest.* 17: 167.
- CONRAD, W. V. & J. H. TALBOT (1940) *J. biol. Chem.* 132: 753.
- CONRAD, W. V. & D. B. DELL (1941) *J. biol. Chem.* 137: 587.
- COPE, C. L. & B. WOLFF (1942) *Biochem. J.* 36: 413.
- CARVER, S. AN (1921) *Biochem. Z.* 123: 304.
- CURRY, A. R. (1920) *J. Physiol. (London)* 52: 991.
- DANOWSKI, T. S. et al. (1955) quoted in ELINGTON, J. R. & T. S. DANOWSKI (eds) *The Body Fluids* (chapter 4). Baltimore.
- DANOWSKI, T. S. (1962) *Personal communication*.
- DARROW, D. C. & A. F. HARTMANN (1929) *Arch. J. clin. Child.* 37: 51.
- DARROW, D. C., E. B. HOPPER & M. K. CARY (1932) *J. clin. Invest.* 11: 701.
- DAUTERMAN, L., H. WHITTON, D. VITZ & J. MEASINS (1933) *Heart* 10: 131.
- D VON, H. W. S. DUCH-BLICH & D. M. MAURICE (1949) *J. Physiol. (London)* 109: 32.
- DAVON, H. (1953) *J. Physiol. (London)* 129: 311.
- DELL, D. B. J. H. TALBOT & H. T. EDWARDS (1930) *J. Physiol. (London)* 69: 267.
- DELL, D. B., H. T. EDWARDS & W. V. CONRAD (1937) *J. biol. Chem.* 118: 631.
- DELL, D. B. J. H. TALBOT & W. V. CONRAD (1937) *J. biol. Chem.* 117: 649.
- DURON, M. N. J. & H. W. MEER (1931) *J. Physiol. (London)* 73: 349.
- DONNAN, F. O. & A. B. HARRIS (1911) *J. chem. Soc.* 9: 1534.
- DONNAN, F. O. (1911) *Z. Elektrochem.* 17: 572.
- DONNAN, F. O. (1924) *Chem. Rev.* 1: 72.
- DONNAN, F. O. & P. JOHNSON (1950) *J. phys. Chem.* 67: 283.
- DOVE, R. F. & P. H. LAYTON (1942) *J. clin. Invest.* 21: 701.
- DRUMER, M. A. GILBY & A. B. HASTINGS (1939) *J. biol. Chem.* 131: 641.

we are inclined to see its usefulness mainly in the fact that from NCE and knowledge concerning the amounts of complex bound cation and anion an estimate of the net charge of the proteins *in vivo* can be made. In normal plasma the difference between NCE and negative net charge is but small and fairly constant owing both to the fact that about equal quantities of filtrable anion and filtrable cation are complex bound and to the relatively constant pH value of plasma. But in pathological states the difference may become far larger because of an increase or decrease in the complex binding of cations relative to that of anions. In view of the clinical use of hypothermia it will be rather interesting to see whether in dialysis experiments performed at a temperature of about 25°C the NCE of the plasma proteins will be found 3 mEq/100 g protein lower as suggested by the experiments of STADIT *et al* (1925) (chapter II), and especially whether the values of the concentration ratios will change in relation to each other.

The most intriguing question however is that concerning the NCE of the non filtrable ionic substances contained within the cells, and its relation to their net charge. Owing to metabolic processes the changes in pH and in the ionic composition inside the cells of active organs must be far greater than those which could ever be observed in plasma *in vivo*. Actually the large changes in membrane potential which have been measured for certain living cells might be caused by large changes in the net charge of the non-filtrable intracellular substances, especially if the pK of their ionogenic groups is in the range 6.5 to 8.0. As a first orientation towards the possibilities in this respect the determination of NCE and of concentration ratios in equilibrium dialysis experiments with intracellular or even mitochondrial material might well prove fruitful. Especially the correlation with pH will be of interest.

## List of Bibliographical References

- ALBERTY R. A. & H. H. MARVIN (1951) *J. Amer. chem. Soc.* **73** 17 2220.
- ALDOREOVICH, H. & C. KORTH (1932) *Deutsche Arch. klin. Med.* **173**, 412.
- ANDER, R., G. CADRE & K. L. ZIEGLER (1956) *J. clin. Invest.* **35** 671.
- ANDER, J. S. & A. S. REILMAN (1959) *Amer. J. clin. Path.* **31** 155.
- ASTRUP F. (1956) *Scand. J. clin. Lab. Invest.* **8**, 33.
- ATCHELEY D. W. R. F. LOER, E. M. BENEDICT & W. M. PALMA (1923) *Arch. intern. Med.* **31** 611.
- ATCHELEY D. W. & E. M. BENEDICT (1930) *J. clin. Invest.* **9** 263.
- AUSCHKE, H. (1874) *Vierteljahrsschrift für Dermatologie und Syphilis* **1** 275.
- BALTZAN, M. A. R. ANDER, G. CADRE & K. L. ZIEGLER (1962) *J. clin. Invest.* **41** 116.
- BARKER, S. B. & W. H. SUMMATION (1941) *J. biol. Chem.* **136** 535.
- BATES, R. O. (1954) *Electrometric pH Determinations, Theory and Practice*. New York.
- BRODMAN, W. M. & J. SNAPPER (1933) *Biochem. Z.* **253**, 195.
- BENJAMIN H. (1932) *Ergänz. zur Med. und Kinder heilende* **42**, 773.
- BRODMAN, L. B. JACOBSON & B. SÄDERHOLM (1963) *Lancet* **1** 992.
- BRODMAN, J. (1962) *Scand. J. clin. Lab. Invest.* **14** Suppl. 66.
- BRODMAN, J. & E. HULTMAN (1962) *Lancet* **1** 1132.
- BREIDENBACH, R. H. (1952) *S. Afr. J. med. Sci.* **17** 101.
- BOEL, A. (1906) *Hyperuræmie als Heilwasser*, 4th ed. Leipzig.
- BURRILL, O. W. (1945) *Amer. J. med. Sci.* **210** 195.
- BUNZMAN, K. D. & B. HAMILTON (1927) *Bull. Hopkins Hosp.* **41** 322.
- BOOT H. L. (1936) *Inleiding tot de fysiologische biochemie*. Hakkert & Loozen.
- BOTH, A. A. (1942) *De bepaling van de zuurfunctie en het quantitative uricae onderzoek*, 4th ed. Hakkert & Loozen.
- BROCH, O. J. (1943) *Acta med. Scand. Suppl.* **166**.
- BROCH, O. J. (1953) *Scand. J. clin. Lab. Invest.* **5** 9.
- BUCHENBERG DE JONG, H. G. G. and associates (1949) in KUTV H. R. (ed). *Colloid Science* vol. **11**. Amsterdam.
- CARR, CH. W. (1933) *Arch. Biochem.* **45** 147.
- CARR, CH. W. (1933) *Arch. Biochem.* **46** 417.
- CARR, CH. W. (1935) *Proc. Soc. exp. Biol. Med.* (N. Y.) **29** 344.
- CARR, CH. W. (1936) *Arch. Biochem.* **62**, 476.
- CHU H. J. & A. B. HARTMAN (1934) *J. clin. Invest.* **17** 167.
- CONRAD, W. V. & J. H. TALBOT (1940) *J. biol. Chem.* **132**, 753.
- CONRAD, W. V. & D. B. DILL (1941) *J. biol. Chem.* **137** 587.
- COTE, C. L. & B. WOLFF (1942) *Biochem. J.* **36**, 413.
- CRIVELLO, S. AN (1921) *Biochem. Z.* **123**, 304.
- CUNY A. R. (1920) *J. Physiol. (London)* **53** 391.
- DANOWSKI, T. S. et al (1955) quoted in ELLINGTON, J. R. & T. S. DANOWSKI (ed) *The Body Fluids* (chapter 4). Baltimore.
- DANOWSKI, T. S. (1962) Personal communication.
- DARROW D. C. & A. F. HARTMAN (1929) *Amer. J. clin. Path.* **47** 51.
- DARROW D. C., E. B. KOPPEL & M. K. CAR (1932) *J. clin. Invest.* **11** 701.
- DAUTERMAN, L. H. WETTERDOR D. VES & J. MALLUM (1923) *Brit. J.* **10**, 133.
- D. VICH, H. W. & DUNCAN-ELDER & D. M. MALROCK (1949) *J. Physiol. (London)* **109** 32.
- D. VICH H. (1955) *J. Physiol. (London)* **129** 111.
- DILL, D. B. J. H. TALBOT & H. T. EDWARDS (1930) *J. Physiol. (London)* **69** 267.
- DILL, D. B. H. T. EDWARDS & W. V. CONRAD (1937) *J. biol. Chem.* **118**, 635.
- DILL, D. B. J. H. TALBOT & W. V. CONRAD (1937) *J. biol. Chem.* **119**, 649.
- DRENN, M. H. J. & H. W. MOOK (1931) *J. Physiol. (London)* **73**, 349.
- DONMAN, F. G. & A. B. HARRIS (1911) *J. chem. Soc.* **99** 1554.
- DONMAN, F. G. (1911) *Z. Elektrochem.* **17** 572.
- DONMAN, F. G. (1924) *Chem. Rev.* **1** 73.
- DORTCHER, R. H. & P. JOHNSON (1956) *J. phys. Chem.* **60** 202.
- DOVE, R. F. & F. H. LAYBETTER (1942) *J. clin. Invest.* **21** 781.
- DRINKER, N. A. A. GREEN & A. B. HARTMAN (1936) *J. biol. Chem.* **131** 641.

we are inclined to see its usefulness mainly in the fact that from NCE and knowledge concerning the amounts of complex-bound cation and anion an estimate of the net charge of the proteins *in vivo* can be made. In normal plasma the difference between NCE and negative net charge is but small and fairly constant owing both to the fact that about equal quantities of filtrable anion and filtrable cation are complex-bound and to the relatively constant pH value of plasma. But in pathological states the difference may become far larger because of an increase or decrease in the complex-binding of cations relative to that of anions. In view of the clinical use of hypothermia it will be rather interesting to see whether in dialysis experiments performed at a temperature of about 25 C the NCE of the plasma proteins will be found 3 mEq/100 g protein lower as suggested by the experiments of STADIE *et al* (1925) (chapter II) and especially whether the values of the concentration ratios will change in relation to each other.

The most intriguing question however is that concerning the NCE of the non-filtrable ionic substances contained within the cells, and its relation to their net charge. Owing to metabolic processes the changes in pH and in the ionic composition inside the cells of active organs must be far greater than those which could ever be observed in plasma *in vivo*. Actually the large changes in membrane potential which have been measured for certain living cells might be caused by large changes in the net charge of the non-filtrable intracellular substances, especially if the pK of their ionogenic groups is in the range 5 to 8.0. As a first orientation towards the possibilities in this respect the determination of NCE and of concentration ratios in equilibrium dialysis experiments with intracellular or even mitochondrial material might well prove fruitful. Especially the correlation with pH will be of interest.

- KIPES, A. (1954) *C. R. Acad. Sci. (Paris)* 239 117
- KLOTZ, J. M. & J. M. URSQUART (1949) *J. phys. colloid Chem.* 53 100
- KRAMER, B. & F. P. TIDWELL (1922) *J. biol. Chem.* 53 241
- LANDOL, E. M., L. SCHAE, M. ANDREYNE & W. EBB (1932) *J. clin. Invest.* 11 717
- LANDOL, E. M. (1934) *Physiol. Rev.* 14 404.
- LEUWEN, A. M. C. M. THOMAS & P. C. KAPTEIN (1941) *Clin. chim. Acta* 6 590
- LEUWEN, A. M. AM. J. J. AN DAATHELAAR & L. A. DE VRIES (1963) *Lancet* 11 94.
- LEWIS, G. N. & M. RANDALL (1923) *Thermodynamics and the free energy of chemical substances* New York
- LEWISLAND O (1916) *Stood Arch Physiol.* 23 153
- LIPPINCOTT, O. & W. SCHODA (1941) *Mitoch. med. Woch* 22 443
- LOEB, J. (1922) *Proteins and the Theory of Colloidal Behaviour* New York.
- LOEB, R. F. D. W. ATCHLEY & W. W. PALMER (1922) *J. Gen. Physiol.* 4 591
- LOEB, R. F. & E. M. BENEDICT (1927) *J. clin. Invest.* 4 33
- LOEWY, A. & N. ZUNTZ (1894) *Pflügers Arch. ges. Physiol.* 58 411
- LOREN, H. F. R. J. RAVEL, O. S. OORDA & S. L. WHITTINGTON (1960) *J. biol. Chem.* 235 3654
- LOVE, W. B. & O. E. BURCH (1953) *J. Lab. clin. Med.* 41 226
- MAJONE, C. L. H. (1946) *Yale J. Biol. Med.* 19 419
- MAJONE, C. L. H. (1947) *J. biol. Chem.* 169 583.
- MATNEY, J. F. (1954) *Physiol. Rev.* 34 334.
- MARRACK, J. (1923) *Biochem. J.* 17 240.
- MARGARIA, R. (1931) *J. Physiol. (London)* 73 311
- MARRINE, T. P. E. E. MICHIGAS, P. JONES & J. M. HILL (1947) *J. Lab. clin. Med.* 32 1208
- MCCLELL, F. C. & A. B. HASTINGS (1933) *J. biol. Chem.* 105, 285
- MCCLELL, F. C. & A. B. HASTINGS (1935) *Ames J. med. Sci.* 149, 601
- MORSE, J. W. (1945) *J. biol. Chem.* 157 173
- MYER, J. W. & P. PACOVISA & R. J. WITZLER (1949) *J. biol. Chem.* 177 13
- NEDERLAND, L. & P. ROMA (1908) *Biochem. Z.* 14 476
- NEUBER, E. C. L. SLEAD, S. LEVINE, J. H. HOLMES & H. ELKRIC (1941) *J. Lab. clin. Med.* 32, 656
- NEUBER, G. A. (1939) *Physiol. Rev.* 19 503
- NOLAN, A. P. & J. Y. COOK (1962) *Lancet* 1 778
- NOLAN, A. P. & J. Y. COOK (1962) *Lancet* 11 133
- NOLAN, B. (1959) *Acta med. Scand.* 165 suppl. 348
- NOLAN, B. S. J. BOYLE, E. J. BIRD, S. D. JACKSON, T. M. BA CHILLOR, L. T. BIRN & O. B. MYERS (1949) *Ames J. clin. Path.* 19 461
- NOLAN, E. C. T. W. & E. POMERANCE (1931) *J. biol. Chem.* 9 732
- NILSSON, B. S. & J. B. PRINCE (1923) *J. biol. Chem.* 57 99
- NORDHO, W. (1933) *Bull. Acad. Pol. Sci. Cl. II Vol III No. 3, 167*
- NORTHROP, J. H. & M. KUNITZ (1925) *J. Gen. Physiol.* 7 25.
- NORTHROP, J. H. & M. KUNITZ (1926) *J. Gen. Physiol.* 9 331
- NORTHROP, J. H. & M. KUNITZ (1928) *J. Gen. Physiol.* 11 481
- ORD, H. C. & J. P. PETERS (1929) *J. biol. Chem.* 81, 9
- OTTELANDER, G. J. A. DEN (1963) Unpublished results. Os. G. A. J. & J. H. M. KOOBMAN-V. EUTEN (1957) *Rec. Trav. chim. Pays-Bas* 76, 590.
- OVERMAN, R. R. & A. K. DAVIS (1947) *J. biol. Chem.* 168, 641
- PAPPENBERGER, J. R. & A. SOTO-RIVERA (1948) *Ames J. Physiol.* 152, 471
- PAPPENBERGER, J. R. (1953) *Physiol. Rev.* 33 387
- PETERS, J. P. H. A. BULGER, A. J. EISENBA & C. LEE (1926) *J. biol. Chem.* 67 141
- PETERS, J. P. A. M. WAKEMAN, A. J. EISENBA & C. LEE (1929) *J. clin. Invest.* 6 517
- PETERS, J. P. A. M. WAKEMAN, A. J. EISENBA & C. LEE (1929) *J. clin. Invest.* 6 577
- PETERS, J. P. & D. D. V. SLYKE (1932) *Quantitative chemical chemistry* Vol. I, Baltimore.
- PETERS, J. P. & E. R. M. (1934) *J. biol. Chem.* 107 33
- PETERS, J. P. (1935) *Study over The exchange of fluids in man* London
- PETERS, J. P. (1932) in DUNCAN, G. (ed), *Doctors of metabolism*, 3rd ed. Philadelphia and London
- PETTERSON, M. A. G. A. FRIED & J. M. CLIMON (1941) *Ames J. Physiol.* 207 346
- PHILIP, R. A. & F. W. PUTMAN (1960) in F. W. PUTMAN (ed) *Plasma Proteins*, Vol. I (chapter 5) New York
- PRASAD, A. S. (1960) *Arch. Intern. Med.* 105, 560.
- PRASAD, A. S. & B. FLINK & R. MC. COLLISTER (1941) *J. Lab. clin. Med.* 34 531
- RABINOWITZ, D. & K. ZIEGLER (1942) *J. clin. Invest.* 41 2191
- RACKENAGEL, K. (1930) *Z. ges. exp. Med.* 68, 439
- RECHTER-QUITTNER, M. (1922) *Biochem. Z.* 126 97
- RODOLPH, L. P. (1933) *De bepaling van het haemoglobine van erythrocyten en de bepaling daarvan naar de kleurloze Tissue Asmetrische*
- ROMA, P. & TAKAHASHI (1911) *Biochem. Z.* 31 336.
- ROMA, E. & H. PEROW (1923) *Biochem. Z.* 137 556.
- ROBERTSON, T. B. (1943) *J. biol. Chem.* 173, 25
- ROBERTSON, E. M. (1944) *J. biol. Chem.* 179 1235
- ROUGHTON, F. J. W. (1933) *Physiol. Rev.* 15 241
- ROUGHTON, F. J. W. (1944) in Walker M. BOOTHBY (ed) *Handbook of respiratory physiology* Project number 21 2304 0083 of the USAF school of Aviation Medicine
- ROUGHTON, F. J. W. (1942) Personal communication.
- SACKNER, M. A. & F. W. SUNDERMAN (1952) *Ames J. Physiol.* 29 17



- EDSALL, J. T. & J. WYMAN (1958) *Biophysical Chemistry* vol. I New York.
- EICHNA, L. W., A. R. BERGER, B. RADER & W. H. BECKER (1951) *J. clin. Invest.* 30 353.
- ELLIOTT, H. C. & H. L. HOLLEY (1951) *Fed. Proc.* 10, 180.
- ERMONT, W. & T. RZEMIAK (1959) *Bull. Inst. mar med (Gdansk)* 10 115.
- ENNEKING, J. H. J. (1956) *De quantitative e libepaling met behulp van papierelectrophorese en haar toepassing bij een onderzoek over proteïnen* Thesis, Amsterdam.
- FANCONI, A. & G. ALAN ROSE (1958) *Quart. J. Med.* 27 463.
- FARMER, S. J. & M. SCHUBERT (1957) *J. clin. Invest.* 36 1715.
- FARHI, L. E. & H. RAHN (1955) *J. appl. Physiol.* 7 472.
- FAHRHOLT, C. (1925) *Journal de chimie physique*, 2 1.
- FAWCETT, J. E. & V. WYNN (1956) *Brit. med. J.* 11 582.
- FEIN, W. O. (1928) *Amer. J. Physiol.* 85 207.
- FERGUSON, J. K. W. & F. J. W. ROUGHTON (1934) *J. Physiol. (London)*, 83 68.
- FERGUSON, J. K. W. & F. J. W. ROUGHTON (1934) *J. Physiol. (London)*, 83 87.
- FISCH, S., S. B. GILSON & R. E. TAYLOR (1950) *J. ppl. Physiol.* 3 113.
- FOLK, B. D., K. L. ZIERLER & J. L. LILIENTHAL (1948) *Amer. J. Physiol.* 153 381.
- FOSTER, J. P. (1960) In F. W. PUTNAM (ed) *The Plasma Proteins*, V. 1.1 (chapter 6) New York.
- GAMBLE, J. L. (1950) *Extracellular fluid* 5th ed., Cambridge, Mass.
- GERBRANDY, J. (1951) *De regulatie van het bloed-plasma volume* Thesis, Amsterdam.
- GERBRANDY, J. H. B., A. HELLENDORN, L. A. DE VRIES & A. M. VAN LEEUWEN (1957) *Scand. J. clin. lab. Invest.* 10 suppl 31 272.
- GERBRANDY, J. A. M. VAN LEEUWEN, H. B. A. HELLENDORN & L. A. DE VRIES (1960) *Clin. Sci.* 19 181.
- GILLMAN, D. R., M. C. VOLK & H. L. BLUMHART (1934) *J. clin. I. vest.* 13 363.
- GRISTINA, G. E. MILLA & R. MANGIARIA (1953) *G. Biochem.* 2 357.
- GLUTCKE, H. J. L. WAYNE & R. GOLDSWORTH (1964) *J. Lab. clin. Med.* 59 40.
- GOLLWITZER-MEIER, K. (1925) *Z. ges. exp. Med.* 46 15.
- GOODMAN, DEWITT S. (1958) *J. Amer. chem. Soc.* 80 3892.
- GOODMAN, DEWITT S. & R. S. GORDON (1958) *Amer. J. clin. Nutr.* 6 669.
- GORDON, R. S. (1955) *J. clin. Invest.* 34 477.
- GORDON, R. S. & A. CHERKES (1956) *J. clin. Invest.* 35 206.
- GORNALL, A. G. CH. J. BARDWELL & M. M. D. VID (1949) *J. biol. Chem.* 177 751.
- GORTER, E. & W. C. DE GRAAF (1955) *Klinische diagnostiek* vol. I 7th ed., Leiden.
- GREENE, C. H., J. L. BOLLMAN, N. M. KEITH & E. G. WAKEFIELD (1931) *J. biol. Chem.* 91 203.
- GREENE, C. H. & M. H. POWER (1931) *J. biol. Chem.* 91 183.
- GROLLMAN, A. (1925) *J. biol. Chem.* 64 141.
- GROLLMAN, A. (1931) *J. Gen. Physiol.* 14 661.
- GURD, F. R. N. & PH. E. WILCOX (1956) *Advances protein Chem.* 11 311.
- GUTMAN, A. B., E. B. GUTMAN, R. JILLSON & R. D. WILLIAMS (1936) *J. clin. Invest.* 15 475.
- GUTMAN, A. B. & E. B. GUTMAN (1937) *J. clin. I. vest.* 16 903.
- HALD P. M. (1933) *J. biol. Chem.* 103 471.
- HALD P. M. & A. J. EISENMAN (1937) *J. biol. Chem.* 118 275.
- HALD P. M. (1946) *J. biol. Chem.* 163 479.
- HALD P. M., A. J. HEINSEN & J. P. PETERS (1947) *J. clin. Invest.* 26 983.
- HALPERIN, M. H. C. K. FREEDMAN & R. W. WILKINS (1948) *Amer. Heart J.* 35 221.
- Handbook of Respiration* (1958) Philadelphia and London.
- HARRIS, K. E. & H. M. MARVYN (1927/29) *Heart*, 14 48.
- HASSELMAN, J. J. F. & E. J. VAN KANPE (1958) *Clin. chem. Acta* 3 305.
- HASTINGS, A. B., D. D. VAN S. YICK, J. M. NEILL, M. HENKELBERGER & C. R. HARRINGTON (1924) *J. biol. Chem.* 60 89.
- HASTINGS, A. B. & J. SENDROY (1925) *J. biol. Chem.* 65 445.
- HASTINGS, A. B., H. A. SALVERSEN, J. SENDROY & D. D. VAN SLYKE (1927) *J. Gen. Physiol.* 8 701.
- HASTINGS, A. B., F. C. MCLEAN, L. EICHELEBERGER, J. C. HALL & E. D. COBT (1934) *J. biol. Chem.* 107 351.
- HENDERSON, L. J. (1931) *Le Sang* Paris.
- HENDERSON, L. J., D. B. DILL, H. T. EDWARDS & W. O. P. MORGAN (1931) *J. biol. Chem.* 90 697.
- HENRIQUES, O. M. (1928) *Biochem. Z.* 200 1.
- HENRIQUES, O. M. (1929) *Ergebn. Physiol.* 25 625.
- HILLER, A., J. PLAZYN & D. D. V. SLYKE (1948) *J. biol. Chem.* 176 1401.
- HOPKINS, TH., J. E. HOW RD & H. EISENBERG (1952) *Bull. Hopkins Hosp.* 91 1.
- HOR TH. S. M. A. RUBIN & E. L. FOLTZ (1940) *Amer. J. Physiol.* 161 316.
- HUCKABEE, W. E. (1958) *J. clin. Invest.* 37 44.
- HUCKABEE, W. E. (1961) *Amer. J. Med.* 30 833.
- HUNTER, T. R. & J. M. KLOTZ (1956) In D. GLICK (ed) *Methods of Biochemical Analysis* Vol. 3 New York.
- INGRAM, R. C., C. LOWBARD & M. B. VINCIG (1932 33) *J. Gen. Physiol.* 16 637.
- JONES, L. J. & D. J. P. KIRBY (1962) *Biochem. J.* 84 152.
- JEANNERET, P. H. ROSENMUND & A. F. ENGEL (1954) *Hel. med. Act.* 21 191.

## ADDENDUM



- SALMIDREN S. (1961) *Studies on the ultrafiltrability of serum sodium and potassium*. Thesis, Helsinki.
- SALVENDY H. A. & G. C. LINDER (1924) *J. biol. Chem.* 58 617
- SALVENDY, H. A. (1928) *Acta med. Scand.* 69 1-6.
- SANDERSON, P. H. (1952) *Biochem. J.* 52, 502.
- SCATCHARD G. A. C. BATCHELDER & A. BROWN (1944) *J. clin. Invest.* 23 438.
- SCATCHARD G. A. C. BATCHELDER & A. BROWN (1946) *J. Amer. chem. Soc.* 68 2320
- SCATCHARD, G. (1949) *Ann. N.Y. Acad. Sci.* 51 660
- SCATCHARD G. I. H. SCHENBERG & S. H. ARMSTRONG (1950) *J. Amer. chem. Soc.* 72 535
- SCATCHARD, G. I. H. SCHENBERG & S. H. ARMSTRONG (1950) *J. Amer. chem. Soc.* 72 540.
- SCATCHARD, G. J. S. COLEMAN & A. L. SEEN (1957) *J. Amer. chem. Soc.* 79 12.
- SCHMIDT C. (1850) *Charakteristik des Epidemischen Cholera gegenüber vermaligen Transmutations-anomalien*. Leipzig und Mitau.
- SEZLES, L. (1936) *Arch. Neerl. Physiol.* 21 526.
- SEVERINGHAUS, J. W. M. STUFFEL & A. F. BRADLEY (1956) *J. appl. Physiol.* 9 189
- SEVERINGHAUS, J. W. M. STUFFEL & A. F. BRADLEY (1956) *J. appl. Physiol.* 9 197
- SHAW L. A. (1927) *Amer. J. Physiol.* 79 91
- SJOGAARD-ANDERSEN, O. K. ENGEL, K. JORGENSEN & P. ASTRUP (1960) *Scand. J. clin. Lab. Invest.* 12 172.
- SJOGAARD-ANDERSEN, O. (1962) *Scand. J. clin. Lab. Invest.* 14 587
- SJOGAARD-ANDERSEN O. (1963) *Scand. J. clin. Lab. Invest.* 15 suppl. 70.
- SOFFER, L. J. D. A. DANTEL, E. B. GROSSMAN, H. SOBOTKA & M. J. JACOB (1939) *J. clin. Invest.* 18 997
- SOFFER, L. J. C. COFFIN E. B. GROSSMAN, M. JACOB & H. SOBOTKA (1941) *J. clin. Invest.* 20 429
- SOULIER, J. P. & J. CROCHIER (1958) *Presse Méd.* 66 617 and 825
- STADIE, W. C. & E. C. ROSS (1925) *J. biol. Chem.* 65 734
- STADIE, W. C. & H. O'BRIEN (1935-36) *J. biol. Chem.* 112 723
- STADIE, W. C. & H. O'BRIEN (1937) *J. biol. Chem.* 117 439
- STRICKLAND R. D. M. L. FRIEDMAN & F. T. GURULE (1961) *Anal. Chem.* 33 545
- SUNDERMAN, F. W. J. H. AUSTIN & J. G. CAMAC (1926) *J. clin. Invest.* 3 37
- SUNDERMAN, F. W. (1930) *J. clin. Invest.* 9 615
- SUNDERMAN, F. W. (1942) *J. biol. Chem.* 143 185
- SUNDERMAN, F. W. (1945) *Amer. J. clin. Path.* 15 219
- SUNDERMAN, F. W. J. F. W. SUNDERMAN, E. A. F. LVO & CH. J. KALLICK (1958) *Amer. J. clin. Path.* 31, 112.
- SWAN, R. C., H. M. FEINSTEIN & H. MADDOX (1956) *J. clin. Invest.* 35 607
- TANFORD CH. (1930) *J. Amer. chem. Soc.* 74 441
- TANFORD, CH. (1952) *J. Amer. chem. Soc.* 74 11
- TANFORD, CH., S. A. SWANSON & W. S. SHORE (1955) *J. Amer. chem. Soc.* 77 6414
- TARAB, R., E. S. HACKER & R. TAYMOR (1912) *J. clin. Invest.* 31 23
- TEREPIKA, A. R., T. Y. TORIBARA & P. A. DEWEY (1958) *J. clin. Invest.* 37 87
- TRUNBERA, T. (1953) *Physiol. Rev.* 33 1
- TORIBARA, T. Y. A. R. TEREPIKA & P. A. DEWEY (1957) *J. clin. Invest.* 36 738.
- TOSTERON, D. C., E. SHEA & R. C. DARLING (1952) *J. clin. Invest.* 31 406.
- TSCHIMMER, H. & C. TSCHIMMER (1924) *C. R. Soc. Biol. (Paris)* 91 392.
- VANLOUQUET B. (1941) *Des Serrueries. Eine plübe-trisch-kütsche und experimentelle Studie*. Thesis, Uppsala.
- VAN SLYKE, D. D. A. B. HASTINGS, M. HEIDELBERGER & J. M. NEILL (1922) *J. biol. Chem.* 54 481
- VAN SLYKE, D. D. H. WU & F. C. McLEAN (1923) *J. biol. Chem.* 56 763
- VAN SLYKE, D. D. & J. M. NEILL (1924) *J. biol. Chem.* 61 523
- VAN SLYKE, D. D. A. HILLER & K. C. BERTHESEN (1927) *J. biol. Chem.* 74 699
- VAN SLYKE, D. D. A. B. HASTINGS, A. HILLER & J. SENDROY (1928) *J. biol. Chem.* 79 769
- VAN SLYKE, D. D. & J. SENDROY (1928) *J. biol. Chem.* 79 781
- VAN SLYKE, D. D. J. R. WESTGATER & K. KILLER (1949) *J. biol. Chem.* 179 746.
- VAN SLYKE, D. D. R. A. PHILLIPS, V. P. DOLL, P. B. HAMILTON, R. M. ARCHERD & J. PLAZEN (1950) *J. biol. Chem.* 183 349
- WALAAS, E. & O. WALAAS (1949) *Acta physiol. Scand.* 17 235
- WALKER, D. B. A. S. PRASAD & J. SADRICH (1962) *J. Lab. clin. Med.* 59 110
- WALKER, M. (1960) *J. clin. Invest.* 39 501
- WALKER, M. (1961) *J. clin. Invest.* 40 723
- WALKER, M. (1962) *J. clin. Invest.* 41 1454
- WAUGH, D. F. (1954) *Advances prot. in Chem.* 11 325
- WILLITS, C. O. M. R. COE & C. L. OGG (1949) *J. Am. off. agric. Chem.* 32 118.
- WILLITS, C. O. & C. L. OGG (1950) *J. Am. off. agric. Chem.* 33 179
- WYMAN, J. (1948) *Advances protein Chem.* 4 406
- ZILSTRA, W. G. & C. J. MULLER (1957) *Clin. chim. Acta* 2, 237
- ZILSTRA, W. G. (1958) *Personal communication*

An analysis (of a plasma sample) is understood to consist of a determination of the value  $x$  and of the various terms in (1) that contribute to the value  $y$  of the sample. The term *experiment* refers to an *in vivo* ultrafiltration experiment, by which a set of samples of various protein contents could be obtained from a single person. Thus, each plasma sample corresponds to a pair of values  $x_{ij}$ ,  $y_{ij}$ , where the subscript  $i$  means the number of the experiment, and  $j$  the number of the sample within the experiment. Actually we had  $1 \leq i \leq 28$ ,  $1 \leq j \leq n_i$ , where  $n_i$  varied from 8 until 13.

2. Selection and arrangement of data. The data which were put at the disposal of the Mathematical Centre for statistical analysis covered 34 experiments. 11 of them fell out since each testee had to be taken into account only once, lest our data become ill-balanced. The remaining 28 experiments could be divided into two groups (denoted by I and II) in a natural way.

The experiments of group I were made in the period from 20-5-60 until 1-12-60, and those of group II from 12-12-60 until 17-11-61; thus, there was some difference of time and of seasons. Moreover the determinations of Na were performed with a different galvanometer.

It was a lucky circumstance that both groups were of the same order of magnitude (see table), since this made them well suited for comparison.

3. Individual regression lines. For each experiment  $i$  (say), Mr VAN LEEUWEN had plotted the values  $y_{ij}$  ( $j = 1, 2, \dots, n_i$ ) against the corresponding values  $x_{ij}$ . The diagrams thus obtained could be explained very satisfactorily by admitting, for each testee a linear regression  $y_{ij} = \alpha_i x_{ij} + \beta_i$  where the parameters  $\alpha_i$  and  $\beta_i$  referring to a single subject, of course may vary with  $i$ .

It was natural, then, to discuss the results on the following simple hypotheses

- (a) The errors of the statistics  $x_{ij}$  may be neglected
- (b) The variates  $v_j$  ( $j = 1, 2, \dots, n_i$ ) are independent with expected values  $E(v_{ij}) = \alpha_i x_{ij} - \beta_i$  for any  $i$  and  $j$
- (c) For any  $i$ ,  $j$  the statistics  $v_{ij}$  are normally distributed with a standard deviation  $\sigma(v_{ij})$  which is independent of  $i$  and  $j$  so we may write  $\sigma(v_{ij}) = \sigma$

(a) was suggested by Prof. HEMELINK. His argument was that computations would be much shortened, whereas the loss of information would be small since  $\sigma(x_{ij})$  is small as compared to  $\sigma(v_{ij})$  (see section 6). Actually he estimated this loss to be less than 10 per cent, which agreed well with the final result. As for (b), the first contention is rather evident, whereas the second was confirmed by the data. Next, by (1),  $y$  is a sum of 5 random terms that are presumably independent. Hence, by the Central Limit Theorem, its distribution will be nearly normal. This holds true under very general conditions; in particular it is unnecessary that each component of  $y$  should itself be normally distributed. As the number of random terms is increased, the convergence of the sum distribution to normal one is fairly rapid, provided the standard deviations of the terms be of the same order of magnitude (their expected values are irrelevant in this respect). Now in our case the standard errors of NaCl and HCO<sub>3</sub> did not differ much. As VAN DER WAAKOP (Verbremaatsche Statistiek, 1957 p. 27) once remarked, 4 is often a big number in Statistics, and here there are always three terms that count (see Le p. 247 for the particular case of the approximation to a normal distribution). So only the assumption of universal standard deviation  $\sigma$  remains open to serious criticism. For the present I pass over possible objections against our statistical model, coming back to it afterwards.

## Addendum

**1 Concepts and conventions.** The following conventions are constantly used. The symbol  $\leq$  means *equal or smaller*.  $b \geq a$  is equivalent to  $a \leq b$ . A bar denotes a mean value, e.g.

$$\bar{x} = \sum_{i=1}^n x_i/n$$

is the mean of the sequence  $x_i$ .

$x$  is a total protein content, expressed in gm.protein/kg  $H_2O$ . Omitting symbols [ ] that denote concentrations (as may be done without giving rise to ambiguity) we define

$$y = Na + K + (Ca + Mg) - Cl - HCO_3, \quad (1)$$

where the 5 observed concentrations at the right (in m.aeq./kg.  $H_2O$ ) are totals including ionized as well as unionized electrolyte.

Unlike values that characterize (in a certain respect) an individual (e.g. length or weight) and which appear to be subject to chance if compared with the corresponding values that mark other individuals of the same kind, *population parameters* are constants that depend only on a population as a whole (or rather that depend equally on all individuals belonging to it) and hence characterize it to a certain extent. Here they are denoted by Greek letters. Examples of parameters are the *population mean* (or *expected value*) the *population standard deviation* and its square usually called the *variance*.

True values of parameters are unknown except sometimes when the population is finite. These can only be approximately determined by calculating *estimates* from samples of the population. Estimates will be denoted by the corresponding Latin letters. Thus, an estimate of the slope  $\alpha$  of a regression line is denoted by  $a$ . In the same way the so-called *standard-error*  $s(x)$  where  $x$  may be any estimate is itself an estimate of the standard deviation  $\sigma(x)$  of  $x$ . The expected value of a random variate is denoted by  $E(x)$ .

In testing a statistical hypothesis by applying it to a particular sample,  $k$  means the *tail probability* of the test statistic ( $T$  say)

In the cases occurring here, this is the probability that  $T$  have a value which (according to the underlying hypothesis) is equal to (or exceeds) the value  $T$  calculated for the sample. A small  $k$  indicates an improbable sample with respect to our hypothesis, and hence is a reason for rejecting the latter. It is common practice to omit the calculation of  $k$  and to decide only whether  $k$  is smaller or bigger than certain preassigned values (0.05 or 0.01 say) that are known as *levels of significance*. Most statistical tables admit of only a comparison of  $k$  with such levels. This, however, gives rise to some loss of information, and hence to less graduated final appraisal.

4. **Comparison of individual results.** The next point concerns the question of whether the calculated regression lines are to be interpreted as particular instances of an ideal regression line, which is valid for all healthy men of medium age, or whether they are (also) noticeably subject to interpersonal variability. The parameters  $a_i$ ,  $\beta_i$  might well depend, e.g. on minute particularities of the constitution of blood proteins. Such individual variations are to be expected: the question is what we can prove about them.

First, the homogeneity of the variances  $\sigma^2$  (or in other words, the independence of  $\sigma^2$  of both  $i$  and  $j$  as was assumed by (c)) can be tested by Bartlett's test (see J. O. KEMPTHORPE, *The design and analysis of experiments* 1952, p. 21). If  $N$  independent variances  $\sigma_i^2$  are given, each of which corresponds to a  $\chi^2$ -distribution with  $n_i - 2$  d. of fr. (as should be true for our estimates  $\sigma_i^2$ ), Bartlett's statistic (a complicated expression, which is here denoted by  $B$ ) is approximately distributed as  $\chi^2$  with  $N - 1$  d. of fr. (evidently  $N - 1$  has nothing to do with the numbers  $n_i - 2$ ). The approximate character of the test need not bother us: it may be that the test can not be relied upon, but only if  $n_i - 2 < 3$  for some values of  $i$ ; in our case we have  $n_i - 2 \geq 6$ , and in all but three experiments even  $n_i - 2 > 8$  (see e.g. J. F. KERRY and E. S. KERRY, *Mathematics of statistics* II, 1951, p. 254 etc.).

The result of Bartlett's test was as follows:

Group	$N - 1$	$B$	$\bar{B}$
I	11	20.6	0.038
II	15	17.2	0.31
I + II	27	40.4	0.047

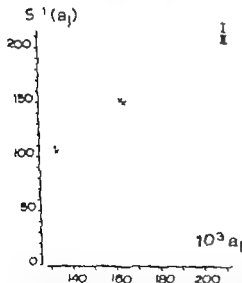
which indicates (rather significantly) that the data of group I (and, consequently, those of the combined groups) deviate from our assumptions. Presumably the hypothesis of 'universal man' must be dropped for group I: it remains uncertain in how far the causes of the inhomogeneity of the variances  $\sigma^2$  in I have been absent in II, in spite of the value  $\bar{B} \approx 0.31$  obtained from Bartlett's test.

Next, in the adjacent diagram, the estimated slopes  $a_i$  have been plotted against the reciprocals  $r^2(a_i)$  of the standard errors. These reciprocals are natural measures of the 'weight' of an estimate: the bigger its standard error, the less trustworthy the estimate itself. Evidently the small slopes on the diagram have only a medium weight, whereas the big slopes are still worse. The reverse, of course, is not true yet, on the whole, the medium slopes are of better quality.

Thus, the statistics  $a_i$  and  $\sigma^2(a_i)$  are, so all appearance, not independent variables. Now it is not clear why this should be so, unless the deviating regression lines are results of experimental errors, too. If we no reason why small fluctuations of  $a_i$  in both directions, should go on to

larger variances  $\sigma^2(a_i)$ , since the larger variances do not appear to be caused by non-linearity of the regression (which could give possible explanation). Hence our diagram is evidence for the view that the true slopes  $\alpha_i$  are less divergent than as indicated by the slopes in the table: it remains uncertain so far, extreme.

Next, the difference of the extreme values  $a_i$  in group II is about 3-4 times the maximum of the numbers  $\sigma^2(a_i)$ : this could hardly be consistent with a normal distribution of the estimates  $a_i$  (and hence, with the hypothesis of practically constant parameters  $\alpha_i$ ). On the other hand, we had



subject	$n_i$	$a_i^2$	$10^3(a_i)$	$10^3 s(a_i)$	$s^1(a_i)$	$b_i$	$s(b_i)$
<u>group I</u>							
vle(l)	13	0.161	145	6.5	153		0.59
Gee	13	0.880	188	14.8	67	5.6	1.33
Ott	12	0.159	173	5.7	176	5.8	0.55
dGe(l)	13	0.598	139	10.2	90	11.4	0.96
Off	13	0.221	162	6.5	163	7.1	0.63
dVr(l)	13	0.590	182	10.8	94	4.6	1.04
Lie	12	0.436	166	7.6	131	5.5	0.85
vdW	10	0.418	133	9.5	106	8.3	0.98
Jan	11	1.132	201	14.5	89	4.3	1.41
Cej	8	1.092	192	16.1	88	3	1.64
Vro(l)	10	0.410	164	8.0	151	5.2	0.0
vdP	12	0.465	143	10.7	94	7.8	1.02

group II

vdR	12	0.154	160	4.7	11	8.4	0.48
Rer	12	0.277	169	7.0	142	4.6	0.67
vSo	12	0.321	136	9.0	111	8.6	0.90
Ste	19	0.300	15	1	141	7.1	0.71
Gou	11	0.433	132	9.1	110	8.0	0.90
Dun	8	0.301	133	8.8	114	7.4	0.85
dRe	11	0.495	184	13.7	73	6.0	1.25
dLo	1	0.98	184	9.8	102	3.4	0.92
Vre	1	0.538	174	10	94	6.1	1.12
Fir	1	1.200	180	1.4	57	6.0	1.65
Poo	1	0.543	1.1	11.3	88	4.7	1.11
Lin	12	0.191	1.2	6.9	145	2.8	0.5
Ilea	8	0.421	191	13.8	72	4.1	1.3
vOp	12	0.353	143	0.8	102	6.5	1.04
Dou	10	0.287	162	11.8	84	4	1.06
Bos	11	0.677	168	11.3	88	6.2	1.13

The fitting of individual regression lines was done by the method of least squares, which, under the hypotheses (a) (b) (c) gives unbiased estimates of  $a_i$   $b_i$  and of their variances  $s^2(a_i)$   $s^2(b_i)$ . For the results, see the table. It follows from the standard errors that the accuracy is somewhat exaggerated (in the computations the accuracy was much more exaggerated in order to avoid rounding-off errors).  $s_i^2$  denotes the estimated variance

$$s_i^2 = \frac{1}{n_i - 2} \sum_{j=1}^{n_i} (y_{ij} - a_i x_{ij} - b_i)^2$$

of the residuals  $y_{ij} - a_i x_{ij} - b_i$  (in Mr v L's text NCE = 100  $a_i$  RA =  $b_i$ ).

For the formulae used see e.g. H. CRAMÉR, *Mathematical Methods of Statistics*, 1946, p. 549-550 (where the standardization of regression lines is somewhat different).

The calculation of regression lines remains valid even if (c) is false, but (c) is very useful since it provides valuable information concerning the distribution of the estimates and their variances, thus enabling us to check some assumptions underlying our model. Thus it follows that  $a_i$  and  $b_i$  are normally distributed and that  $(n_i - 2)s_i^2/\sigma^2$  has a  $\chi^2$ -distribution with  $n_i - 2$  degrees of freedom (abbreviated d. of fr.).

**TABLES BELONGING TO**

**NET CATION EQUIVALENCY**  
**( BASE BINDING POWER )**  
**OF THE PLASMA PROTEINS**

*by*

**A. M. VAN LEEUWEN**



no res  
well k  
the at-  
in two  
assum  
Fin  
analy  
If T  
cent f  
line, l  
sump

B No .....

**S M S MEDICAL COLLEGE LIBRARY**  
**DUE DATE SLIP**

*This book is to be returned on or before the date  
marked below —*

**A fine of annas four will be charged for each day  
the book is kept overtime**

5. Weigher  
of weights  
weight of  
somewhat

where  $\bar{a}$  is  
the value  
quite sati  
weighed  
corrective

6. Additi  
the varia  
the defin  
group I  
meanum  
Mr V  
formula

--	--	--	--

ver it is  
ston that  
explained  
as, or by

applying  
for group  
he 0.1 per  
regression  
basic as

duction  
about the  
neral be

ement of  
but still  
nt of the  
e for the

bution of  
We had  
those of  
ad in the

lying the

where  $\Lambda$  ...  
any pair of corresponding observations. The asterisk refers to actual variances. From  
his data  $s^{*2}(x) = 0.1331$   $s^{*2}(y) = 0.0873$ . By assuming the values  $x_{ij}$  to be exact we  
add  $\alpha^2 \sigma^2(x)$  to the variance  $\sigma^2(y)$ . Of this additional term we have an estimate  $\alpha^2 s^{*2}(x) =$   
 $= 0.0034$ . Hence Prof. HEMELRIJK's argument.

Mathematical Centre, Amsterdam

C. G. G. VAN HIRK

**TABLES BELONGING TO**  
**NET CATION EQUIVALENCY**  
**( BASE BINDING POWER )**  
**OF THE PLASMA PROTEINS**

*by*

**A. M. VAN LEEUWEN**



substrate	method	n	pH range	NCK at pH 5	NCK increase per unit pH increase	authors
				mEq per	100 g prot	
			7 7 7	17.3	0.8	Van Slyke et al. (1923)
				20.3	0.7	Hastings et al. (1927)
			6.5 7.0	27.3	12.	
				18	7.7	
			6 6.0	22.3	16	Van Slyke et al. (1928)
		ten D (chapter II)		23.8	18	
			7.0 7.5	27.3	1	Darrow Hartman (1925)
			6 6.5		18.8	Hammarum et al. (1921)

30 number of samples utilized

# E R R A T A

For explanation asterisks see page 131 in text.

table 2

CO<sub>2</sub> not in mEq/L. but in mL/L.

table 3

protein concentrations in g/Kg H<sub>2</sub>O

table 4

number of samples from which correlation between v is content and protein concentration was calculated.

## EXPLANATION

The tables are indicated by two numbers the first is encircled and represents the actual number of the table the second number refers to the page in the text on which the table is first mentioned . For example

⑬ 69      means      table 13   first referred to on page 69



(Sodium) or (Potassium)  
 (Formate) or (Malate)  
 number of subjects

protein  
 g/L

# CATIONS

Na K Ca Mg

mmoles  
 cellulose  
 bag

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

1 1 1 1

# ANIONS

Cl HCO<sub>3</sub>

mmoles  
 pH

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

1 1

# METHODS

Na  
 total  
 base

# REFERENCES

- Guarise 1954  
 Krane & Tinsall 1922  
 Murray 1923  
 Salmons & Lunde 1924  
 Salmons et al. 1926  
 Peter et al. 1926  
 Salmons 1926  
 Salmons 1926  
 Card & Peter 1929  
 Durrey & Hartmann 1929  
 Atchley & Benedict 1930  
 Salmons 1930  
 Dill et al. 1930  
 Dill 1931  
 Dill & Klemm 1931  
 Dill et al. 1931  
 Casseljo & Talbot 1940  
 Casseljo & Talbot 1940  
 French 1943  
 French 1943  
 French 1943





substance	concentration mEq per litre plasma	ref. no.	further remarks
$\text{SO}_4$	0.7	87 mEq/L. Loeb & Benedict (1937) 83 mEq/L. Mill et al (1961)	
lactate	0.8	0.78 mEq/L. Andre et al (1956) 0.64 mEq/L. Huckabee (1964) 1.04 mEq/L. Huckabee (1961)	after 1 hr complete rest recovery period (in bed)
non-esterified fatty acids	0.6	Gordon & Cherkas (1956)	
citrate	0.3	Thunberg (1953)	
keto acid	0.2	Rosenthal (1949)	

The values given for lactate refer to blood



substance	concentration mEq per litre plasma	references	furthe remarks
$\text{SO}_4$	0.7	0.7 mEq/L. Loeb & Benedict (1927) 0.8 mEq/L. Mill et al (1951)	
lactat	0.8	0.78 mEq/L. Andre et al (1956) 0.8 mEq/L. Mackabee (1958) 1.0 mEq/L. Mackabee (1961)	ft 1 hr complete rest recovery period (in bed)
non-esterified fatty acid	0.6	Gordon & Cherkas (1958)	
citrate	0.3	Thomberg (1953)	
keto acid	0.3	Rosenthal (1949)	

The values given for lactat refer to blood

	prot		Na		M		Ca+Mg		Cl		CO <sub>2</sub>	
	g / L				m m q p		l l					
temp. de l'eau (°C)	21	22	21	22	21	22	21	22	21	22	21	22
in vitro ultrafiltration I	82.8	82.8	122.5	122.5	4.1	4.04	8.81	8.78	104.4	104.6	24.9	24.3
	82.7	82.7	122.6	122.6	4.09	4.06	8.84	8.84	104.3	104.8	24.9	24.9
	82.8	82.7	122.9	122.8	4.18	4.15	8.83	8.84	102.8	97.9	24	24.7
	82.9	84	122.5	122.5	4.25	4.21	7.98	8.2	10	4	29.5	23.1
in vitro ultrafiltration II									101.3	102.2	24	23.9
									102	102.6	24.7	24.7
									10	5	10	9
									98	100.5	24.5	24

	<u>small tube</u>		<u>large tube</u>	
	Cl	CO <sub>2</sub>	Cl	CO <sub>2</sub>
	mEq / L		mEq / L	
for consecutive sampl from an in vivo ultrafiltration experiment	92.9	27.6	92.7	27.6
	97.9	29.9	97.9	29.7
	97.4	29.3	97.6	29.3
	97.4	29.3	97.3	29.3

sampl	CO <sub>2</sub>	
	<u>tube</u>	<u>syringe</u>
	mEq / L	
1	29.9	30.2
2	30.2	30.2
3	29.8	29.6
4	29.6	29.4
5	29.6	29.6
6	29.2	29.3
7	29.6	27.0
8	29.6	29.4

	P	I	Kjeldahl
	Kjeldahl	Kjeldahl	nitrogen
	g P	g I	g N
<u>plasma from normal subjects (in vivo ultrafiltration technique)</u>			
uL	73.3	72	0.0
	67.7	63.6	0.2
	87.6	84	0.3
V	110.7	1.9	0.2
Bo	62.2	63.0	1.0
	87.0	87.2	2.2
	100	102	-0.1
<u>plasma from patients (blood plasma) serum protein or albumin</u>			
	8.7	8.8	0.1
3	9	70.1	0.2
3	64.3	62	0
	76.9	76.3	0
	84.6	3	0.2
6	77	77.0	-0.2
	76.3	76.3	0.2
<u>human serum albumin</u>			
	78.1	78	0.2
<u>plasma from patients</u>			
Bo (blood vs 300 mg/L)	5.2	57	-0.7
uL (myeloma)	27.1	27.6	0.6
Bo (myeloma)	82	82	2
Bo (myeloma)	88	86.7	
	84	87.7	2.8
	88.3	68	2.6
Note indicates plasma samples diluted with saline solution			

	P	F	I	I		
<u>pairs of duplicate determinations</u>						
	27	13	85	10	82	61
<u>concentration range (g/L)</u>						
	84	84	33	82.5	97	104
	84	72	83	82	104	114
SD of single determination in plasma (g/L)	0.28	0.28	0.43	0.23	0.27	0.20
SD of mean of duplicate determination (g/L)	0.25	0.20	0.37	0.23	0.19	0.20

m i p l i t t a	p H b l	d
	exp. 1	exp. 2
6 ml blood drawn anaerobically into syringe	7.340	7.308
after adding 2 ml air: gentle mixing and expulsion of air	7.344	7.304
After adding another 2 ml air repeated pulling out plunger to the 10 ml mark with syringe capped, and expulsion of air	7.350	7.305

	p H b l	d	% HbO <sub>2</sub> blood	
	exp. 1	exp. 2	exp. 4	exp. 5
directly from syringe	7.343	7.333	39	41
after transfer to testtube	7.344	7.355	38	38

Note testtube filled beforehand with alveolar air and closed with rubber cap

	w e i g h t			
triplet of triplicet det. rminations range of wet content (g/L)	67	68	66	
	980 930	930 910	910 840	
SD of singl det rmination in plasma (g/L)	0.41	0.43	0.44	
SE of the mean of triplicet determination (g/L)	0.24	0.26	0.23	

sample	1	2	3	plasma minus stated plasma		deproteinized plasma minus plasma	
				Na	K	Na	K
	prot g/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
1		5	4.50	0.0	0.0	3.0	0.21
2		20.0	0.0		1	2.0	0.00
3		132.3	0.0		0.0	1.0	0.2
4		2.0		-0.2	0	0.0	0.05
5		127.8	4.15	0	-0.02	2.0	0
6		171	4	0	-0.7	-0.2	0
7		127	2.00	-0.0	0.23	1	0.00
8		127	2.31		-0.00		0.11
9		100		0.0			
10		20	0.0	0	0.0	2.2	-0.20
11		46	2.00	0	0.0		0.00
12		140.9		0			
13		22		0.0			
14		27.0		-0.2			
15		20		0			
16		4.2	37	0.2	0.07		
17		0.4	30		-0.20		
18		2	30	-0	0.10		
19		0.4	20.0		-0.20		
20		0			-0.2		
21		20.0	12.0	0.1	-0.21		
22		0.4	22	-0.2	-0.21		
23		0.4	15		-0.22		
24		0	2	0	-0		
25		0.2	20.3	-0	-0.14		
26		0.2	22	-0	0		
27		0.0	0.0	0.0	-0.07		
28		0.0	0.0	0.0	0.0		
29		0.0	0.0	0.0	0.0		
30		0.0	0.0	0.0	0.0		
31		0.0	0.0	0.0	0.0		
32		0.0	0.0	0.0	0.0		
33		0.0	0.0	0.0	0.0		
34		0.0	0.0	0.0	0.0		
35		0.0	0.0	0.0	0.0		
36		0.0	0.0	0.0	0.0		
37		0.0	0.0	0.0	0.0		
38		0.0	0.0	0.0	0.0		
39		0.0	0.0	0.0	0.0		
40		0.0	0.0	0.0	0.0		
41		0.0	0.0	0.0	0.0		
42		0.0	0.0	0.0	0.0		
43		0.0	0.0	0.0	0.0		
44		0.0	0.0	0.0	0.0		
45		0.0	0.0	0.0	0.0		
46		0.0	0.0	0.0	0.0		
47		0.0	0.0	0.0	0.0		
48		0.0	0.0	0.0	0.0		
49		0.0	0.0	0.0	0.0		
50		0.0	0.0	0.0	0.0		
51		0.0	0.0	0.0	0.0		
52		0.0	0.0	0.0	0.0		
53		0.0	0.0	0.0	0.0		
54		0.0	0.0	0.0	0.0		
55		0.0	0.0	0.0	0.0		
56		0.0	0.0	0.0	0.0		
57		0.0	0.0	0.0	0.0		
58		0.0	0.0	0.0	0.0		
59		0.0	0.0	0.0	0.0		
60		0.0	0.0	0.0	0.0		
61		0.0	0.0	0.0	0.0		
62		0.0	0.0	0.0	0.0		
63		0.0	0.0	0.0	0.0		
64		0.0	0.0	0.0	0.0		
65		0.0	0.0	0.0	0.0		
66		0.0	0.0	0.0	0.0		
67		0.0	0.0	0.0	0.0		
68		0.0	0.0	0.0	0.0		
69		0.0	0.0	0.0	0.0		
70		0.0	0.0	0.0	0.0		
71		0.0	0.0	0.0	0.0		
72		0.0	0.0	0.0	0.0		
73		0.0	0.0	0.0	0.0		
74		0.0	0.0	0.0	0.0		
75		0.0	0.0	0.0	0.0		
76		0.0	0.0	0.0	0.0		
77		0.0	0.0	0.0	0.0		
78		0.0	0.0	0.0	0.0		
79		0.0	0.0	0.0	0.0		
80		0.0	0.0	0.0	0.0		
81		0.0	0.0	0.0	0.0		
82		0.0	0.0	0.0	0.0		
83		0.0	0.0	0.0	0.0		
84		0.0	0.0	0.0	0.0		
85		0.0	0.0	0.0	0.0		
86		0.0	0.0	0.0	0.0		
87		0.0	0.0	0.0	0.0		
88		0.0	0.0	0.0	0.0		
89		0.0	0.0	0.0	0.0		
90		0.0	0.0	0.0	0.0		
91		0.0	0.0	0.0	0.0		
92		0.0	0.0	0.0	0.0		
93		0.0	0.0	0.0	0.0		
94		0.0	0.0	0.0	0.0		
95		0.0	0.0	0.0	0.0		
96		0.0	0.0	0.0	0.0		
97		0.0	0.0	0.0	0.0		
98		0.0	0.0	0.0	0.0		
99		0.0	0.0	0.0	0.0		
100		0.0	0.0	0.0	0.0		

Note indicates the sample from patients with myeloma when these also are left out we calculate from the samples with known protein content the (K) having these values

protein content of samples		g/L
Na ('plasma minus stated plasma')		-0.11 to 0.11 g/L
K ('plasma minus stated plasma')		-0.10 to 0.10 g/L



	plasma	fraction I	plasma fraction I
albumin	37.3	0.0	37.3
$\alpha_1$ and $\beta$ -globulin	18.4	1.0	32.0
fibrinogen	2.0	11.2	14.0
$\gamma$ globulin	10.2	5.3	12.5

all values re to g/L

Difference between N and K values found  
with 'deproteinised plasma' and those found with 'plasma'

number of samples		67	61	55
protein concentration	<del>mean</del> range g/L	68.6 60 to 80	61.1 50 to 100	110.0 100 to 125
N ('deproteinised plasma minus 'plasma')	<del>mean</del> range mEq/L	2.1 0.6 to 2.9	2.6 0.8 to 3.6	2.0 1.1 to 4.9
K ('deproteinised plasma minus 'plasma')	<del>mean</del> range mEq/L	0.13 -0.13 to 0.30	0.10 -0.13 to 0.31	0.14 0.03 to 0.40

samples	pH	m		'plasma minus shed plasma'		'deproteinized plasma minus 'plasma'		
		prot g/L	Na	K	Na	K	Na	K
			mEq/L		mEq	/L	mEq	/L
1			25.6	99	0	1	2	0.21
2			136.5	96	0.9	-0.1	2	0.0
3			26.2	3.96		0.0	0	0
4			2.6		-0.2		2	-0.1
5			172	4		-0.2	-0.2	0
6			177.0	7		-0.7	1.0	0.96
7			24.1	2.56	-0.1	-0.22	1.0	1.0
8			27.4	2.90	0	-0.00	1.0	1
9			179.5		0.5	-0.00		
10			1.5			0.00	4	-0.20
11			29.1	4.00		0.00	2	0.66
12			144	2.70				
13			40.9		0			
14			122		-0.9			
15			0		1			
16			126.4		-0.2	-0.10		
17			122	4.20				
18		4.5	157	80	0.2	-0.07		
19		10.4	20.3	4.0	0.0	-0.30		
20		72	20	4.30	-0.1	-0.10		
21		0.4		0.0	1	0.30		
22		3.0	4.70		0.1	-0.1		
23		126	123	80	0.1	-0.1		
24		64.2	22	2.9	-0.2	-0.2		
25		94	15.1			0.22		
26			1	4.0	0.2	-0.1		
27			4.1	7.0	-0.1	-0.1		
28			22	4	-0.1	-0.2		
29		6.4		2.2		-0.07		
30		6.2	4.0	4.1		0.0		
31		0.04	4.0	4		-0.05		
32		2.1	4.0	0	0.0	0.02		
33		7.0	2.4	9.2	-0.1	0.0		
34		0.7	2.2	2.00	-0.1	-0.1		
35		14.2	2.2	0.0	-0.1	-0.04		
36		0.2	2.0	0.0	0	-0.1		
37		0.2	2.0	2.96		-0.00		
38		0.4	2.0	4	-0.1	-0.04		
39			2.0	0	-0.1	-0.1		
40		1.2	2.0	2.54		-0.1		
41			2.0	2.54		-0.1		
42		0.7	2.0		1	0.0		
43		0.5	2.0	2.2		-0.12		
44		0.4	2.0	2.2		-0.1		
mean also				0.10	-0.00			
maximum value						0.0		
minimum value						-0.22		

Note indicates the samples from patients with myeloma when these values are left out calculate from the samples with known protein content (the following mean also)

protein content of samples 57.0 g/L  
 Na ('plasma minus 'shed plasma') -0.11 mEq/L  
 K ('plasma minus 'shed plasma') -0.00 mEq/L

plasma sample	apparent prot. in conc. g/l.	$\frac{Na}{K}$ measured m/m expected	$\frac{K}{Na}$ measured m/m expected
I	35	0.2	0.02
(N content 1.2 mEq/100 g prot.)	73	0.1	0.04
(K content 0.08 mEq/100 g prot.)	144	-0.2	-0.12
	216	1.2	-2.16
II	63	0.1	
(N content 1.1 mEq/100 g prot.)	189	-0.5	
III	30	0.0	
(N content 0.0 mEq/100 g prot.)	72	0.1	
	108	-0.3	
	144	-0.6	
	180	1.2	
IV	30	0.0	
(N content 1.1 mEq/100 g prot.)	60	0.2	
	90	0.4	
	120	0.6	
V	180	0.0	
(N content 0.0 mEq/100 g prot.)	180	0.0	
	180	0.0	

Note: the values given for plasma V represent the results of three separate experiments. The apparent protein concentration referred to the protein concentration in the solutions analysed but is expressed as the protein concentration of hypothetical plasma which in 1:100 dilution would have the same protein concentration as the solution actually analysed.

consecutive determinations on one sample of plasma	$\frac{m}{m} \frac{E}{E} \frac{A}{A}$			$\frac{m}{m} \frac{K}{K} \frac{B}{B}$	
	N	K		Na	K
	mEq	p		li	
1	132.3	4.93		132.6	5.18
2	132.6	4.97		132.6	4.90
3	132.7	5.00		132.9	4.95
4	132.4	5.00		132.6	5.27
5	132.4	5.00		132.6	4.95
6	132.3	5.01		132.6	5.00
7	132.7	5.00		132.6	5.03
8	132.5	4.97		132.3	5.03
9	132.6	4.97		132.3	5.12
10	132.7	5.01		132.9	5.03
mean value	132.52	5.006		132.26	5.016
maximum value	132.7	5.18		140.4	5.27
minimum value	132.3	4.93		132.6	4.83

	$\bar{X}_n$	$\bar{X}_w$
pair of duplicate determinations concentration range (mEq/L)	642	642
SD of single determination in plasma (mEq/L)	1.80	1.41
SD of the mean of duplicate determinations (mEq/L)	0.13	0.11
	0.06	0.06
	$\bar{X}_n$	$\bar{X}_w$
pair of duplicate determinations concentration range (mEq/L)	1001	1001
SD of single determination in plasma (mEq/L)	2.0	2.0
SD of the mean of duplicate determinations (mEq/L)	0.17	0.17
	0.07	0.07
or 1/2 the SD of the mean of duplicate determination		calculated at 0.07

protein analyzed against BSA control	NaCl standard mM	protein mM	absorbance at 280 mμ	uncorrected mg/L	corrected for NaCl in solvent
I Lysozyme 4.5 g/L	0.5		1	100	100
		0.5		0.2	20
		0.5	7	100	100.1
	0.5	1.0	7	100.0	99.1
	0	2.0	7	0.1	100.2
II Lysozyme 30 g/L	0.5		7	00.0	00.0
		1.0	7	0.0	0
	0.5	0	7	00.0	100.0
	0.5	2	7	10.0	99.0

	plasma	deproteinized plasma
1	104	104
2	97	97.3
3	94	104
4	93	104.2

#### bio-mimicking experimental procedure

[illegible]

plasma samples	apparent prot in conc g/L	Na measured minus expected mEq/L	K measured minus expected
I	36	-0.2	0.02
(N content 1.2 mEq/100 g prot)	72	-0.3	0.04
(K content 0.05 mEq/100 g prot)	144	0.6	0.12
	216	1.9	-0.16
II	63	0.1	
(N content 1.1 mEq/100 g prot)	129	0.6	
III	36	0.0	
(N content 0.0 mEq/100 g prot)	72	0.1	
	108	0.3	
	144	-0.6	
	180	1.3	
IV	30	0.0	
(N content 1.1 mEq/100 g prot)	60	0.3	
	90	0.6	
	120	0.6	
V	180	0.0	
(Na content 0.0 mEq/100 g prot)	180	0.0	
	180	0.0	

Note The values given for plasma V represent the results of three separate experiments. The apparent protein concentration refers to the protein concentration in the solutions analyzed but is expressed as the protein concentration of a hypothetical plasma which in 1:100 dilution would have the same protein concentration as the solution actually analyzed.

consecutive determinations on one sample of plasma	method A		method B	
	N	K	Na	K
	mEq p		lit	
1	139.3	4.03	132.6	0.10
2	139.8	4.07	132.6	4.90
3	139.7	5.00	137.9	4.85
4	139.4	5.00	142.4	5.27
5	139.4	5.00	139.0	4.95
6	139.3	5.01	140.0	5.00
7	139.7	5.19	138.8	5.03
8	139.8	4.97	138.2	5.05
9	139.6	4.97	138.2	5.12
10	139.7	5.01	138.9	5.05
mean value	139.57	5.004	132.29	5.040
maximum value	139.7	5.19	140.4	5.27
minimum value	139.3	4.93	132.6	4.90

	<u>Na</u>	<u>K</u>
pairs of duplicate determinations	543	543
concentration range (mEq/L)	120 144	24 36
SD of single determination in plasma (mEq/L)	0.2	0.011
SE of the mean of duplicate determination (mEq/L)	0.03	0.003

	<u>C</u>	<u>Ca+Mg</u>
pairs of duplicate determinations	509	504
concentration range (mEq/L)	2 8	4.5 9
SD of single determination in plasma (mEq/L)	0.017	0.017
SE of the mean of duplicate determination (mEq/L)	0.003	0.003

or Mg the SE of the mean of duplicate determination was calculated as 0.017

serum dialysed against dist. water	NaCl standard solution	serum	diluted NaCl in 50% ethanol	measured Ca	corrected for Cl in serum
	ml	ml	ml	mEq/L	
I	5			100	99.6
(protein 43 g/L)		0.5	7	0.2	0.0
	0.5	0.5	7	99.5	100.1
	0	1.0		99.9	99.4
	5	2	7	1.1	100.2
II	5			99.0	99.0
(protein 36 g/L)		1.0	7	0.0	0.0
	0	1.0	7	99.0	100
	0.5	2.0	7	1.0	99

sample	plasma	deproteinised plasma
	mF	/L
I	94	94.0
II	92	92.3
III	102.5	100
IV	101.5	98

Note concerning experimental procedure

plasma 5 ml plasma ml (1) or centrifugation ml added to  
and ( 2% 1% gelatin) titration with AgNO<sub>3</sub>  
deprot plasma 5 ml plasma 2 ml (2) ml 1% ribonucleic acid  
after centrifugation an supernatant added to ml ( 2%  
1% gelatin) ration pH AgNO<sub>3</sub>  
standard solutions treated in the same manner slope of titration curve  
and point of equivalence not altered in the presence of ribonucleic acid  
residue blank the same for plasma and for deprot plasma

plasma sample	apparent protein conc g/L	$\frac{Na}{K}$ measured min expected mEq/L	$\frac{K}{Na}$ measured min expected
I	36	0.3	-0.03
(Na content 12 mEq/100 g prot)	72	0.3	0.04
(K content 0.05 mEq/100 g prot)	144	0.3	0.12
	216	1.3	0.16
II	63	0.1	
(Na content 1.1 mEq/100 g prot)	126	-0.6	
III	38	0.0	
(Na content 0.9 mEq/100 g prot)	72	0.1	
	108	-0.2	
	144	-0.6	
	180	1.3	
IV	30	0.0	
(Na content 1.1 mEq/100 g prot)	60	-0.3	
	90	0.4	
	120	0.6	
V	180	0.0	
(K content 0.0 mEq/100 g prot)	180	0.0	
	180	0.0	

Note: the values given for plasma V represent the result of three separate experiments. The apparent protein concentration referred to the protein concentration in the solutions analysed but is expressed as the protein concentration of a hypothetical plasma which in 100 dilution would have the same protein concentration as the solution actually analysed.

consecutive determinations on one sample of plasma	method A		method B	
	Na	K	Na	K
	mEq/g	g	111	
1	132.3	4.93	132.6	4.18
2	132.6	4.97	132.6	4.90
3	132.7	5.00	132.9	4.85
4	132.4	5.00	140.4	5.27
5	132.4	5.00	132.9	4.93
6	132.3	5.01	140.0	5.00
7	132.7	5.18	132.6	5.05
8	132.6	4.97	132.3	5.05
9	132.6	4.97	132.3	5.12
10	132.7	5.01	132.9	5.05
mean value	132.52	5.004	132.26	5.049
maximum value	132.7	5.18	140.4	5.27
minimum value	132.3	4.93	132.6	4.83

		$\text{HbO}_2$ %	$\text{F}_{\text{O}_2}$ mm Hg	pH	$\text{P}_{\text{CO}_2}$ mm Hg
precompression	<u>mean</u> range	83 (78-88)	36	7.312 (7.31-7.40)	46.3 (39-56)
endcompression*	<u>mean</u> range	10 (7-22)	15	7.294 (7.28-7.37)	54.4 (48-62)
postcompression	<u>mean</u> range	55 (38-81)	31	7.347 (7.33-7.39)	48.6 (39-62)

Note: endcompression refers to values obtained from 25 to 48 minutes after the start of compression.

		number of observ	pH decrease during compression	
			mean	range
van LEEUWEN et al (196 )	normal subjects and patient	52	-0.05	-0.02 to -0.09
present study	normal subjects	24	-0.04	0.00 to -0.10
	patients	28	-0.05	-0.02 to -0.13

Note: pH decrease of more than 0.08 was only observed in three instances twice it was 0.1 once 0.12

	<u>lactate (venous blood)</u> $\text{mEq/Lg H}_2\text{O}$	
	mean	range
precompression	1.04	0.82 to 2.10
compression ( 0 to 20 min)	1.5	1.00 to 2
compression (20 to 48 min)	1.56	1.04 to 2.62
postcompression	1.23	0.87 to 2.18

Precompression value minus postcompression value

protein	pH	$\text{Na}$	$\text{K}$	calcium	$\text{Cl}$	$\text{HCO}_3$	lactate	(calcium minus $\text{Cl} + \text{HCO}_3 + \text{lactate}$ )
$\text{g/Kg H}_2\text{O}$		mEq	mEq	mEq	mEq	mEq	$\text{H}_2\text{O}$	
1	0.01	0	-0.00	-0.00	-0.06	0.23		0.25



containe	mp l hours after start dialy 1	N		Cl		Valhard	
		flam mEq/L	potent tit mEq/L	N minus Cl		mEq/L	Na minus Cl
I	0	183.3	183.3	0.0		183.3	-0.1
	16	183.7	183.8	-0.1		183.4	1.7
	24	183.7	183.8	0.1		183.3	1.2
	40	183.6	184.4	-0.8		183.3	+0.3
II	0	183.3	183.3	0.0		183.7	+0.8
	16	183.3	183.3	0.0		183.6	0.8
	32	182.9	183.2	-0.3		183.1	+0.8
	40	183.1	183.4	-0.3		182.7	+0.4
III	0	184.7	184.8	0.1		184.2	0.5
	16	184.7	184.4	+0.3		184.8	0.1
	24	185.1	185.3	0.1		183.8	1.3
	40	184.6	183.0	0.4		183.3	1.4
IV	0	182.8	182.4	+0.2		180.8	2.1
	16	182.8	182.6	-0.1		180.8	1.6
difference between N and Cl conc			m ange +0.3	0.13 t -0.8		+0.57 2.1	1.7

subject and sample	prot in g/Kg H <sub>2</sub> O	Mb					Mb g/l
		α <sub>1</sub> gl	α <sub>2</sub> -gl	β-gl	γ-gl	percentage of total prot in	
Do 1	72.7	82.7	2.7	6.1	8.8	21.3	
	79.3	82.2	2.1	6.5	6.8	21.4	
	83.1	82.3	2.5	6.4	6.9	21.8	
	87.7	82.8	2.3	6.6	7.2	21.2	
	112.2	82.0	2.8	10.0	7.0	21.6	
	113.2	82.0	4.2	10.0	6.7	20.1	
	86.1	82.6	2.4	8.9	7.3	21.7	
	77.1	82.1	2.9	8.2	6.8	21.1	
	73.7	82.2	4.0	8.9	6.8	21.1	
II	67.8	82.2	2.4	7.1	7.2	20.1	
	67.8	80.4	2.8	7.1	7.8	21.0	
	80.1	80.4	2.7	7.0	7.8	21.3	
	93.7	80.7	2.8	7.6	7.3	20.9	
	108.2	82.8	2.7	7.9	8.4	20.1	

		$\text{HbO}_2$ %	$\text{P}_{\text{O}_2}$ mm Hg	pH	$\text{P}_{\text{CO}_2}$ mm Hg
precompression	<u>mean</u> range	83 (29 88)	39	7.343 (7.31 7.49)	46.3 (39 64)
endcompression*	<u>mean</u> range	18 (7 33)	15	7.294 (7.26 7.32)	84.4 ( 8 62)
postcompression	<u>mean</u> range	94 (79 95)	31	7.347 (7.32 7.38)	45.8 (35 63)

Note endcompression refers to values obtained from 25 to 40 minutes after the start of compression.

		number of subjects	<u>pH decrease during compression</u>	
			mean	range
van LEEUWEN et al ( 54 )	normal subjects and patients	53	-0.05	0.02 to -0.09
present study	normal subjects	34	-0.04	0.00 to -0.10
	patients	28	-0.03	-0.02 to -0.13

Note pH decrease of more than 0.03 was only observed in three instances  
times was 0.04, 0.05, 0.13

	<u>lactate (venous blood)</u>	
	mEq/Kg	$\text{g}^{\circ}$
	mean	range
precompression	1.50	0.82 to 2.10
compression (10 to 30 min)	1.89	1.04 to 2.40
compression (30 to 60 min)	1.68	1.04 to 2.62
postcompression	1.33	0.67 to 2.18

<u>precompression value minus postcompression value</u>									
protein g/Kg $\text{H}_2\text{O}^{\circ}$	pH	$\text{Na}$	$\text{K}$	anions mEq / Kg	$\text{Cl}$	$\text{HCO}_3$	lactate $\text{H}_2\text{O}$	{anions minus $\text{Cl}$ $\text{HCO}_3$ "lactate" }	
0.1	0.1	-0.40	-0.40		-0.06	0.03		0.23	

oestane	mpl	hours aft start dialy	N		Cl	
			name	potent. tit	V l/hand	
			mEq/L	mEq/L	N mEq/L	Cl mEq/L
I		0	153.3	153.3	0.0	153.3
		16	153.7	153.8	-0.1	153.4
		24	153.7	153.8	0.1	152.5
		40	153.6	154.4	0.8	153.3
II		0	153.3	153.3	0.0	152.7
		16	153.3	153.3	0.0	152.5
		24	152.9	153.3	-0.3	152.1
		40	153.1	152.4	0.3	152.7
III		0	154.7	154.6	0.1	154.2
		16	154.7	154.4	+0.3	154.8
		24	155.1	155.3	-0.1	153.8
		40	154.6	155.0	0.4	153.2
IV		0	152.6	152.4	+0.2	150.5
		16	152.5	152.6	-0.1	150.9
diff. range between N and Cl conc			mean range		0.13 +0.2 to 0.8	+0.57 2.1 to 1.7

subject and exampl	protein g/Kg H <sub>2</sub> O	alb	$\alpha_1$ g/l	$\alpha_2$ g/l	$\beta$ g/l	$\gamma$ g/l
		percentage of total prot				
Do 1	72.7	59.7	2.7	8.1	7.3	21.3
	78.2	59.2	2.1	8.5	6.0	21.4
	82.1	58.3	2.5	8.4	6.0	21.0
	87.7	58.8	2.3	8.8	7.2	21.2
	112.2	58.0	2.5	10.0	7.0	21.6
	113.2	58.0	4.3	10.0	6.7	20.1
	113.2	58.6	2.4	8.9	7.2	21.7
	86.1	58.1	2.9	8.2	6.6	21.1
	77.1	58.2	4.0	8.8	6.8	21.1
Bo 1	67.8	62.2	2.4	7.1	7.2	20.1
	67.8	60.4	2.8	7.1	7.8	21.0
	80.1	60.4	2.7	7.0	7.8	21.3
	93.7	60.7	2.5	7.8	7.3	20.9
	106.2	59.8	2.7	7.9	8.4	20.1

SD of single determinations of  $\Delta_{\text{Na}}$ ,  $\Delta_{\text{K}}$  and  $\Delta_{\text{tot}} \text{ mEq Cl} \cdot \text{HCO}_3^-$

(valid for comparison of results obtained from different containers)

the values are calculated for dialysis units containing 63 g/Kg  $\text{H}_2\text{O}$  protein

SD in mEq/100 g protein

$\Delta_{\text{Na}}$	$\frac{100}{87} \sqrt{(0.03)^2 + (0.11)^2 + 0.22^2}$	0.06
$\Delta_{\text{K}}$		0.06
$\Delta_{\text{Ca}+\text{Mg}}$		0.10
$\Delta_{\text{Cl}}$		0.03
$\Delta_{\text{HCO}_3^-}$		0.33
$\Delta_{\text{tot mEq Cl} \cdot \text{HCO}_3^-}$		0.91

difference between values obtained for  
"distilled water" and for dialysis solution (Urea sample taken)

pairs of samples		mean differences	SD of the differences for single pair	range of differences
pH	73	0.03	0.1	-0.10 to 0.13
mEq / Kg $\text{H}_2\text{O}$				
Na	73	-0.02	0.1	-0.2 to 0
K	73	-0.001	0.02	-0.06 to 0.07
Ca Mg	53	-0.001	0.03	-0.14 to 0.04
Cl	73	-0.04	0.10	0.7 to 0.4
$\text{HCO}_3^-$	7	-0.01	0.37	-0.8 to 0.7
tot mEq $\text{Cl} \cdot \text{HCO}_3^-$	71	0.3	0.42	-0.7 to 1.7

difference between value obtained from two samples of the same dialysis solution (sample taken first in sequence minus sample taken second)				
	pairs of samples	mean difference	SD of the differences for single pair	range of difference
pH	22	0.003	0.01	-0.02 to 0.04
			mEq / Kg	H <sub>2</sub> O
Na	22	0.08	0.28	0.8 to 0.8
K	22	0.01	0.030	-0.04 to 0.12
Ca Mg	22	-0.01	0.025	0.08 to 0.03
Cl	22	0.01	0.20	-0.4 to 0.8
HCO <sub>3</sub>	22	0.03	0.22	0.5 to 0.5
(calculated) Cl HCO <sub>3</sub>	22	0.03	0.40	0.7 to 0.6

difference between value obtained from two serum containing dialysis salt (same container) (sample taken first in sequence minus sample taken second)				
	pairs of samples	mean difference	SD of the differences for single pair	range of difference
pH	48	-0.003	0.01	-0.03 to 0.03
			mEq / 100 g	l
ΔN	54	0.03	0.81	1.7 to 2.2
ΔK	54	0.008	0.08	-0.03 to 0.11
ΔCa Mg	54	0.010	0.10	-0.22 to 0.24
ΔCl	54	-0.10	0.77	1.8 to 1.9
ΔHCO <sub>3</sub>	54	-0.03	0.44	-0.8 to 1.0
Δ(calculated) Cl HCO <sub>3</sub>	54	0.21	0.80	1.7 to 2.0

Note: the actual protein concentrations of the serum samples ranged from 8.2 to 13 g/Kg H<sub>2</sub>O the mean value being 11 g/Kg H<sub>2</sub>O

dialysis solution	pH	Na	K	Ca+ Mg	Cl	NaCO <sub>3</sub>	inorg. phos.	urea nit	urea am	osm. mole lit
		m	Eq	Eq	g	g	mg	g	g	
before dialysis	7.68	147.7	8.96	4.3	126.6	28.9	1.78	187.2	187.3	-0.3
after dialysis										
container I										
sample 1	7.91	147.1	8.96	4.28	126.7	28.3	1.74	186.7	186.7	0.0
sample 2	7.91	147.6	8.94	4.28	126.8	28.4	1.74	186.8	186.9	-0.1
container II										
sample 1	7.83	147.1	8.96	4.28	126.9	28.7	1.73	186.4	187.3	-0.9
sample 2	7.83	147.6	8.94	4.28	127.0	28.9	1.83	186.8	187.6	-0.8

samples	pH	protein g/kg H <sub>2</sub> O	inorg. phos. mg/kg H <sub>2</sub> O
dialysis sol.	7.73	—	1.00
serum	7.4	83.6	1.00
dialysis sol.	7.3	—	1.00
serum	7.13	83.1	1.00
dialysis sol.	6.98	—	1.30
serum	6.55	84.6	1.34
dialysis sol.	7.04	—	16.8
serum	7.83	31.4	16.3
serum	84	82.6	16.6
dialysis sol.	7.15	—	16.6
serum	7.80	31.6	16.3
serum	7.80	82	16.3
dialysis sol.	7.71	—	31.9
serum	7.73	22.9	31.3
serum	7.73	84.1	36.7

sample in the sequence	pH	E	P	I	Exp II pH	Exp III pH
		CO <sub>2</sub> mEq/L	P	CO <sub>2</sub> mm Hg		
1 dialysis solution	7.72	32.8	27		7.09	7.61
2 'distilled wat	7.72	32.8	27		7.10	7.51
3 'distilled wat	7.72	32.8	27		7.08	7.51
4 'distilled wale	7.72	32.8	27		7.08	7.58
5 dialy is solution	7.72	32.7	27		7.08	7.51
6 'distilled water	7.74	32.9	28		7.10	7.52
7 dialysl solution	7.72	34.2	27		7.08	7.81
8 'distilled wat	7.72	32.8	27		7.08	
9 dialysis solution	7.73	32.7	26		7.08	

protein dialys mls	g/Kg H <sub>2</sub> O	ΔNa		ΔCa Mg		ΔCl (t ml HCO <sub>3</sub> )	
		pl	—	pl	—	pl	—
		mEq / 100		g p		t i	
dialysis xp I	83 to 89	91	93	2.74	2.71	1.71	1.24
		91	91	2.62	2.68	1.65	1.22
dialysis xp II	83 to 87	8.8	8.4	2.22	2.08	1.52	1.29
		8.4	8.6	2.28	2.04	1.51	1.27
mean vals		8.8	8.9	2.47	2.16	1.60	1.26

dil t d m ml			also obtained with y dil t d serum			
protein concentration			mean vals obtained with dil t d serum			
range mean mmls			ΔCl		ΔCl (min HCO <sub>3</sub> )	
g/Kg H <sub>2</sub> O			mean difference	2 SE	mean diff reverse	2 SE
			mEq / 100		g p	
40 to 50	43.8	9	-0.98	0.74	0.44	0.77
30 to 40	33.7	18	0.88	0.68	0.76	0.71
20 to 30	25.3	8	0.28	1.71	0.22	1.80
10 to 20	16.8 18.3	6 5	2.18	2.40	2.40	2.80

Not for use of th mplex in the protein range (10 20) HCO<sub>3</sub> was no det remind

dialysis solution	pH	Na m	K m	Ca Mg mg	Cl F %	HCO <sub>3</sub> %	inorg phos mg %	am acid %	urea %	res min %
before dialysis	7.88	147.7	8.88	4.37	128.8	28.9	1.78	187.2	197.8	-2.3
after dialysis										
container I										
sample	7.91	147.	8.86	4.36	128.7	28.3	1.74	186.7	196.7	8.8
sample 2	7.91	14.8	8.04	4.36	128.8	28.4	1.74	186.8	196.8	-2.1
container II										
sample	7.89	14.1	8.08	4.36	128.8	28.7	1.73	186.4	1.3	-4.9
sample 2	7.93	147.8	8.04	4.36	127.8	28.9	1.88	186.8	187.6	-2.8

sample	pH	protein g/Kg H <sub>2</sub> O	inorg phos mEq/Kg H <sub>2</sub> O
dialysis sol.	7.7	—	1.80
serum	7.74	62.6	1.89
dialysis sol.	7.13	—	1.96
serum	7.13	53.1	1.95
dialysis sol.	8.98	—	1.98
serum	8.96	54.5	1.94
dialysis sol.	7.04	—	1.66
serum	8.2	51.6	0.3
dialysis sol.	7.04	54.5	1.66
dialysis sol.	7.15	—	1.85
serum	7.38	28.8	1.83
serum	7.38	66.4	1.83
dialysis sol.	7.71	—	2.15
serum	7.73	28.8	2.15
serum	7.73	66.1	20.7



32 128

samples in th l sequence	pH	E p l		Exp. II	Exp. III
		CO <sub>2</sub> mEq/L	P CO <sub>2</sub> mm Hg		
1 dialysis solution	7.72	33.8	27	7.09	7.51
2 'distilled wat	7.72	33.8	27	7.10	7.51
3 'distilled water	7.72	33.8	27	7.09	7.51
4 'distilled wat	7.72	33.8	27	7.09	7.50
5 dialysis solution	7.72	33.7	27	7.09	7.51
6 distilled wat	7.74	33.8	25	7.10	7.52
7 dialysis solution	7.72	34.2	27	7.08	7.51
8 'distilled wat	7.72	33.8	27	7.08	
9 dialysis solution	7.73	33.7	28	7.08	

33 133

	g/Kg H <sub>2</sub> O	prot m	Δ Na		Δ Ca Mg		Δ (Cl + HCO <sub>3</sub> )	
		dialysis	pl	se	pl	se	pl	se
		ratio	mEq / 100 g		g		t	
dialys exp. I	88 to 89		8.1	8.2	3.74	3.71	17.1	15.4
			8.1	8.1	2.83	2.68	16.5	16.2
dialys exp II	83 to 87		8.5	8.4	3.22	3.08	13.3	15.9
			8.4	8.6	3.25	2.84	13.1	14.2
mean value			8.8	8.9	2.97	2.86	16.0	15.5

34 135

			value obtained with u dil d serum			
dil t d	m	mpl	mean value obtained with dil l d serum			
protein concentration			$\Delta Cl$		$\Delta (Cl + HCO_3)$	
range	mean	number	mean difference	2 SE	mean difference	2 SE
g/Kg H <sub>2</sub> O			mEq / 100 g			
40 to 50	43.8	8	0.08	0.74	0.44	0.77
30 to 40	35.7	18	-0.86	0.68	0.78	0.1
20 to 30	25.5	5	0.28	1.71	-0.22	1.80
10 to 20	16.8	6	3.18	2.40		
	16.3	5			2.40	2.68

Not fit one of the sample in the protein range (10 to 20) HCO<sub>3</sub> was determined



date	subject	age	number of samples	protein lab value (%)	pH plasma	water content plasma	equation	PLASMA CO CPNTR							TIO
								N	OLF	mp	Ca	Mg	Cl	NaCl	in ph
					percentage	percentage		prot	Na	K	Ca	Mg	Cl	NaCl	
					percentage	percentage		g/L	mEq	p	p	p	l		
20-0-00	vLo 87	37	1	1	7.37	795-0.0	03	5		89					
20-0-00	Cho	3	3	53	99	884-0.5415	03	5	77						
21-0-00	Cho	37	2	73	7.34	955-0.537	03	3	1	179					
21-0-00	dCho 66	72	1	77	36	39	99-0	P 06	67	36.6	38		64	95	75
2-0-00	Cho		3	6.8	7	30	996-250 P 03			99			72	87	36.4
2-0-00	dV 85	63	1		37	7.39	909	39	3.3	34	2	4	50	6	22.2
16-0-00	Lie	33	1		35		997	799	03	1	34	1	4	94	3.8
0-0-00	vdf	3	1	5.5	36	3	994	55	16	4	37	288	83	96	73.3
-1-00	an	3			7.34		998	86	P 03	64.3	36	3.53	47.5		7.5.3
17-11-00	C J	83		53	34	38	994	P 06		94	88	4	75	95.9	26.3
24-4-00	ro 03	22		96	20	32	997	775	03		54	19	4		3
3-13-00	vdf	29	2	68	7.3	7.33	52-0.767	06	64	64	73	78	54	86	26
3-13-00	vdf	2	2	8	7.36	7	994-0.797	06	64	74.8	73	3	96	85	25
1-0-0	ro	38	3	73	3	7.29	997-0.523 P 06			4	7		84	36	
3-0-01	vLo	38	2	33	7.25	7.27	995-0	P 06		37			84	3	63
3-0	Se	38	3	3	7.37		994	798 P 03	64	4	6	54	73	88.6	36
1-0-0	Cho	34	1	35	7	4	994	P 03		36.6	39	62	52		24
3-0-01	Duo	30	5	64	9	32	997-0.876 P 06		37			64.2	64	84	34
1-0-01	dLo	35	1	64	37	1	994	694 P 03	71.3	3		72	76	85	24.3
8-0-01	dLo	38	2	7.94	7	2	999-0.622 P 03	4	3		39		93	89	24
3-0-0	ro	32	2	75	38	39	994	77 P 03	96	35.7	1	6.87	84	27	1
1-0-0	Si	34			5		997	0.54	03	87	1	1.2	3.75	64	62
30-0-0	Puo	25	3	73	27	34	996	88	64.8		5		73	87	30
26-0-0	Lie	36	2	90		32	994	03	76.6	76	3.77	76	87		24
1-0-01	Cho	5	8		26	38	89	8.66	88	62.3	95.3	64	53	64	84
1-0-0	Opp	38			7.34		995-0.864 P 06	73	34	366			97	1	24
1-0	Duo	3	1	5.5	38	36	997	779	06	64.8	2.9	23	84	54	27
17-0-0	Se	31	1	64	7.55	26	996-74 P 06	63	64	94					27
mean value					7.28		996	28 P	78	3	31.3	3.8		84	24
maximum value					98	23			79	3	33			94	24
minimum value						27			94					73	27
0 0 1 0 0 0 1 0 0 1 1															
1-00	Lo 25			70	38	39				36				75	88
3-0	Lo 13						98	53	88		24				25
0-0	Lo 30			77	40				64	37				84	87
0-0	dV 35				36	39	89	78	03	64	37	79	34	94	27
29-0-0	dCho 03			58		38			76	39	99			64	28
29-0-0	ro 03			72	39				64	37				64.2	

	pH	m	N	K	Ca	Mg	Cl	NaCl	leucoph
	protein	protein	m	m	m	m	m	m	m
experimental result	g/l plasma	g/l plasma	g/l plasma	g/l plasma	g/l plasma	g/l plasma	g/l plasma	g/l plasma	g/l plasma
number observations	(20)	(20)	(20)	(20)	(27)	(27)	(20)	(20)	(20)
mean value	68	7.30	138	2.85	4.65	88	104.0	29.1	1.0
maximum value	76.5	7.40	2	4.22	88	130	109.5	30.5	2.5
minimum value	61.3	7.21	28	2.78	4.7	7.8	101.7	22.2	1
correction mean value									
for 17740			-0.4	0.1					
d)		0.03						0	
best estimate									
for 17740 value	68.2	7.30	29	2	4.65	88	104.0	29.1	1.0

protein composition (mean values in % of total protein)

Alb 58 (a 27)      alb 57      gamma glob 10      gamma glob 26.8  
 beta glob 20      beta glob 20      beta glob 20      beta glob 20

but (ref 1) ) ) d) etc refer to the section on systematic errors  
 in chapter V

	pH	m	N	Ca	Mg
	protein	free	bound	free	bound
	mEq per 100 g H <sub>2</sub> O	mEq per 100 g H <sub>2</sub> O	mEq per 100 g H <sub>2</sub> O	mEq per 100 g H <sub>2</sub> O	mEq per 100 g H <sub>2</sub> O
experimental result	(20)	(20)	(27)	(27)	(27)
mean value		46.3	94	2	20
maximum value	2	1.9	1.5	2.1	1
minimum value	27		94		19
correction mean value					
for 17740		-0.2	-0.3	-0.04	-0.02
d)	0.3	0	0	0	0
best estimate					
for 17740 value	24	134.8	9.2	2.75	1

Note the corrections marked with \* are very much an estimate for their representation the reader is referred to chapter IV

vals obtained when the regression line is calculated from

subject	<u>all samples</u>		<u>compression sample only</u>	
	RA	NCE	RA	NCE
	mEq per Kg H <sub>2</sub> O	mEq per 100 g prot	mEq per Kg H <sub>2</sub> O	mEq per 100 g prot
Geo	3.8	14.8	4.6	17.8
Ott	5.7	17.4	5.5	17.6
dGe (2)	11.5	13.8	13.0	12.9
Off	7.1	18.2	8.0	17.3
Ren	4.6	16.9	6.0	14.9
dV (2)	7.6	14.1	4.9	14.4
dGe (2)	5.3	18.7	4.6	18.4
dR	8.9	18.4	4.2	20.0
dLo	3.4	18.4	3.1	18.7
Vre	6.3	17.2	8.0	15.0
Le (2)	4.4	17.2	3.5	18.0
Blr	6.0	18.0	4.4	18.1
Poo	4.7	17.0	2.8	18.8
Lin	2.8	17.2	1.8	18.0
Hex	4.1	18.2	2.6	20.4
Dow	7.5	16.2	6.6	17.9
See	6.1	16.9	2.6	19.1
mean value	5.6	17.0	5.0	17.7

error caused by addition of heparin

heparin solution added to samples	N		RA	N	
	plasma conc.	free		bound	NCF
	mEq / Kg H <sub>2</sub> O			mEq/100 g prot in	
0.01 ml	+0.2	+0.1	+0.1	+0.1	+0
0.02 ml	+0.6	+0.3	+0.3	+0.2	+0.2

	1		2		3		4		5		index plate
	Protein g/L plasma	pH Preconcentr	Na mEq/L	K mEq/L	Ca mEq/L	Mg mEq/L	Cl m	SO <sub>4</sub> m			
<u>experimental results</u>											
number observations	(28)	(28)	(28)	(28)	(27)	(27)	(28)	(28)	(28)		
mean value	68.2	7.36	172.3	2.53	4.5	6.8	104.6	26.1	138		
maximum value	76.8	7.46	1.3	4.32	4.89	9.6	108.3	26.9	2.5		
minimum value	6	7.2	3.6	2.3	4.3	6.8	1.1	22.3	1.4		
<u>correction mean value</u> <u>for effect</u>											
			-0.6	0							
		0.07						0			
<u>best estimate</u> <u>of mean value</u>	68.2	7.3	6	9.6	4.5	1	104.6	26.1	1		

protein composition (mean values in % of total prot in)

albumin (a 7) 55% pseudo-glob 16.4% glob 28.5%

electrophoresis (a 29) albumin 56% of 7% of 7.4% glob 6.3% glob 1.8%

Note: error a) ) ) d) are refer to the section on systematic errors in chapter

	1		2		3		4		5	
	pH concentration	free mEq per Kg H <sub>2</sub> O	% bound	free mEq per 100 g prot	% bound	free mEq per Kg H <sub>2</sub> O	% bound	free mEq per Kg H <sub>2</sub> O	% bound	free mEq per 100 g prot
Experimental results										
Number observations	(28)	(28)	(28)	(27)	(27)	(27)	(27)	(27)	(27)	
mean value	1	46	0	2.94	2.78	2.9	0.87			
Maximum value	27	43	1	7	2.1	1.5				
Minimum value		1		2.64	2	0.2				
Correction mean value for effect		-0.2	-0.6	-0.05	0.07	-0.03	0.02			
	0.1	-0.2	-0.7							
Best estimate of mean value	1	46	0	2.94	2.78	2.9	0.87			

Note: the corrections marked with \* are very much an estimate for their argumentation the reader is referred to chapter IV

	pH	RA	NCE
	endcompr	mEq per Kg H <sub>2</sub> O	mEq per 100 g prot
<u>experimental result</u>			
number observations	(26)	(28)	(28)
mean value	7.31	6.2	18.4
maximum value	7.35	11.5	20.2
minimum value	7.27	2.8	12.2
<u>correction mean value</u> for error			
b)		-0.2	-0.2
c)		0.8	0.2
d)	0.03	-0.1	0.7
e)		0.1	0.3
<u>best estimate</u> of mean value	7.34	6.0	17.8

	year	number observations	protein g/Kg H <sub>2</sub> O	cation III plasma mEq per Kg H <sub>2</sub> O	free silicon mEq per Kg H <sub>2</sub> O	bound silicon mEq per 100 g prot	free silicon percentage plasma conc.
	1961	18	71.0 <sup>oo</sup>	2.02	2.92	2.98	53
<u>Ca</u>	present study	27	72.9	4.95	2.94	2.78	59
	1961	18	71.0 <sup>oo</sup>	1.78	1.12	2.88	64
<u>Mg</u>	present study	27	72.9	1.79	1.20	0.83	67

Not van LEEUWEN et al. (1961)

<sup>oo</sup> protein concentration 3% too low because of error in macro-Kjeldahl determination (see chapter III)





date and subject	sex	age	diagnosis	number of subjects	protein (g/L)	immunoglobulin (g/L)	pH plasma	ASMA					ONCENT AT I				other substance
								M	C	Hg	Cl	HC <sub>2</sub>	Na <sub>2</sub> P <sub>2</sub> O <sub>7</sub>	Na <sub>2</sub> SO <sub>4</sub>	I	E	
								g/L	g/L	g/L	g/L	g/L	g/L	g/L	g/L	g/L	g/L

24.1	F	73	myeloma (γ-type)	30	7.34	7.34		12.0	3.54	80	1.09	91.0	3	172				
21-24	Ja	84	myeloma (γ-type)		3.734			14.0	134	2.76	6.77	1.04	84	34.8	244			
22-24	Re	84	myeloma (γ-type)	21	12	2.30	7.34	11.0	12.9	37	31	1.00	10	2	86	86		
17-24	Re	84	myeloma (δ-type)	23	7	5	40	86.3	18	1	80	193	94.7	253				
23-24	Ja	84	myeloma (γ-type)	23	7.30	7.30		130	126	37	6.99	84	58.0	72				
27-24	Ja	84	myeloma (δ-type)	0	23	1	7.34	20	11.0	36.3	4.95	1.79	20	22.9	100			

Prep in m

0.00	re	84	liver cirrhosis		1	5	30	36	70	1	3	63	86	100	22	144		
1-1-00	Ja	84	subacute glomerulonephritis	20	0	30	30	27	36		1	27	104	2	3		renal	24
20-18	Ja	86	chronic infection	23	1	27	32	70	36		1	84	99.6					
4-1	Ja	86	chronic infection	20		7.34		72	12.9			1	25					
4-24	Ja	86	hypernephroma	4	3	34	7.30	23	32		81	70	100	26.1	81			
20-24	Ja	86	liver cirrhosis	27	4	40		90	6.3	23	54	5.2	47	3	2			
1-44	Ja	87	liver cirrhosis	80	2.3	25	7.30	68.6	24.7	4	4	1	94.7	34				
24-44	Ja	87	kidney metastases	20	7.30	23		6	18	60	27	27	102	34	72			
1-4	Ja	88	Waldenström's disease	5		29	24	74				1.00	2	23				
1-5-00	Re	87	subacute glomerulonephritis		2.40	36		52.3	30.7	09	1.44	94	34	8		renal		
6-5-00	Re	87	subacute glomerulonephritis	20		70	33		31	1.9	08	80	104	22	27		renal	9

Prep in

1-5-00	Re	84	chronic glomerulonephritis	20		7			3	86	30	2	95		1.30		renal	27
23-1-00	Ja	88	chronic glomerulonephritis	20		30		75	24	86	54		4	1.57		renal	27	
23-1-00	Ja	88	chronic glomerulonephritis	27	0.3	21	29	71		92	27		96		75		renal	27
24-1-0	F	86	chronic glomerulonephritis	80	0.3	27		72		37	70	27	94				renal	27
1-5-0	Ja	86	chronic glomerulonephritis	20	2	3	30		76	92		100		85		renal	27	
1-5-00	Ja	86	chronic glomerulonephritis	27		30			22.5	00	23	23	76		84		renal	27

Prep in

24-20	Ja	87	subacute glomerulonephritis	20		70		73	24	23		62	84	24	39			
21-44	Ja	84	chronic glomerulonephritis	3		37		37		70		84	21	22				
24	Ja	75	subacute glomerulonephritis	20	2.44	36		30	27	60	1.00		36	28				
4-1-00	Ja		subacute glomerulonephritis	20		2		70	30	40	70	104	24					
20-2-78	Ja	44	subacute glomerulonephritis	20	30	30		71	24	21	23	102	27	34				
0.00	Ja	87	subacute glomerulonephritis	20		21	30		21.0	30	00	92	84	20	20			

Notes: Indicate hyperproteinemia in which the end test analyzed in hyperproteinemia plasma renal stands for creatinine

[illegible]

DMST

W R I Y S O I I O

Number daily not used  
(Pillars) or (Syringes)

I

date	subject	pH	at main CH <sub>3</sub> PCl <sub>2</sub>		pH	at main HClO <sub>2</sub>		Na	K	Ca mg	Cl	HCO <sub>3</sub>	Number daily not used (Pillars) or (Syringes)	pH	per d	HCl	Δ Na	Δ K	Δ Ca	Δ Cl	Δ HCO <sub>3</sub>														
			m Kg	per yr		m																													
							M T W T F S S I Y S O I I O																												
2-1-66	Lo B	37	2		7.56			3		3		36	3 P	54.3					37	37															
2-1-66	J		1		40	3		56		36		36	3	4.3		24		29	29	1.4															
2-1-66	re B				7.24	2		1.36		1	9	3.2	P	70	86			34																	
2-1-66	nd				56	7				96	4	36		7.56	8	22			35																
2-1-66	vdp		2		6			1.66		82	136	37	3 P	6.3		21		31	3.86																
2-2-66	vle B		1		36	1		1	12	2.05	6	3.2							59																
														0.5					79																
2-4-66	pool I		2							2.02		2	3 S	7.2		6			1.76																
										2		3.2						36																	
					36					37		5.3	3	7.5		6		36	64																
16-6-66	Om		-0.8		64	2		64	1.63	1.86									34																
16-6-66	Lo B		66		6					62									33																
			64		1							22.3							64																
			62		6					47		26	3 S	6.4		6.6			64																
2-6-66	Dom		6.3		2.36			3		2		27.0							66																
			6		0.63					2.99	26		3						64																
2-6-66	vle B		37		36	1		63	2	6	37	26							24																
					79			63		6	17.1	22.3	3 S	6		29	56.5		37																
4-6-66	vle-Lo		36	2.6						1.96	6.1	31	26						37																
			62	2.6		6.56				1.99	6	22	24						37																
			62		66					6.3	6.36	26	26						64																
5-6-66	dy		66		66					6.79	6	26							64																
										6.36	6	27	27						79																
			91	1.3	6.86					77	6	26	27	1 S	6	66	67		64																
24-6-66	vdp		72		67					6.77	6.69	2	26.3						6.36																
			6.54		36					23	6.67	36	26						27																
			66		36					77	6.66	34	25.3	3 S	6	66	6		6.36																
25-6-66	dy		66	-0.8	63	1				6.67	26	26							37																
										72	6	26	26	3 S	6	66	2		64																
										79	6	26	26						64																
6-6-66	dSa		7.64		66					6.6	66	6.69	21	36					64																
			67		66					6.6	66	6		36					64																
			63		63					6.6	67		29	23					64																
6-6-66	dLo									6.2	79	6	21	26					64																
			6.3	1.0	6.37					79	6	27							64																
6-6-66	re		7.33		36					66	62	26.3							64																
			7	3	64					6.62	21	26	3 S	6.3					64																
			79		79					74	6	26	22						64																
9-6-66	re		7.23		36					6	66	26							64																
			7	3						6.62	27	27							64																
2-6-66	re B		64		66					76	62	26	6						64																
					36					72	6	26	26						64																
2-1-66	pool II		66		66					26	76	63	27						64																
			7		76					29	76	67	27						64																
					76					66	76	26	23						64																
26-6-66	pool II		7.62		62							26							64																
			36		23					66	62	26							64																
			6.66		66					72	62	21	22						64																
3-1-66	pool IV		7.96		67					62	67	22	27						64																
			7.62	6.2	66					63	66	24	27						64																
										62	66	24	27						64																
a t i e n t																																			
2-1-66	Fin		Male		0																														
			6.67																																
			6.3																																
			23																																
26-1-66	vO		Male		73 yr																														
			7.73																																
			6.54																																
13-1-66	Pa B		Male		67 yr																														
			63																																
			7.57																																
			63																																
22-1-66	Pa B		Male		63																														
			7.66																																
			7.12																																
			64																																
27-2-66	Lo		Female		66 yr																														
			76																																

[illegible]



[illegible]

date	subject	pt	sal in Ct-H <sub>2</sub> O ml Kd	pt	at mos HCO <sub>3</sub> m	K	Ca Mg	Cl	HCO <sub>3</sub>	salinity (‰) P.T. (‰) (‰)	pH	total H <sub>2</sub> O H <sub>2</sub> O	HCl	Δ	Δ	Δ
11-00	vLd II	27	2	7		3	67	2	36	3	36	64	23	6	27	2
21-00	J				2.2	2	68		36			44	3	36	36	1.95
21-00	re II	29		3		43.0	38		3.2	7	39	64		6	31	1.95
1-12-00	WCP			60		7	2	80	34			56	23		32	
1-12-00	vLd II	2		28		2	40	72	28	3		47	1	1	27	2.98
27-34	vLd II	33		30	-0.6	3.52	39		22	7	47	63	1	1	1	94
4-6	pool I	45		3	2	3		92	63	3		60	1	1	30	18
				62		60		97								
				30		3.3		92				60	1		34	94
16-64	Om		-0.5	44		4	53	90	2				1			
8-64	vLd II	10	-0.5	45		2			3	8	50	4	2		33	33
		6.09	3.8		1	0	50		122	21		54	1			
									20	36		54	62		6	
8-64	Don	6	6	30		2		75	2	2	5	1.11	1	3	30	34
		6	6	95		7		80	126		2	5	8			
8-64	La II	27	2	2.98		45.6	3.95		3	5		54			74	1
				670		45.3			117	23		54.5			2	3.37
6-64	vLd-La	30	2	27		3.64		12	28.9	2		54			54	
		6.00	2	50					122	34		54			54	
		6.64	2	95		3	53	30	130	94		6.64	60	24		47
11-64	dy	60				5	479	1	10			70	27	2	16	77
		6.5	1	80		1	77	1	25	27		64			74	
24-64	reW	72	2	67		3	477	96	1	10.3		64			74	
		7.64		30		3	477	97	30	24		64			74	
		90			3.2	2.2	473	98	24	1		64	6	24	74	80
25-64	dCo	7		82	1	2	4	92	90	24		7.0	0	20	72	00
		7.64	-0.6	99	-0.6	4.78		92	22	10		64			77	71
						4.78		25.8	25							00
10	dCo	7.04	1.3		1.3	6.8	4.99	99	0	36		64	62	14	54	
		87		84		64	4.82		24.8	36		64	62		73	85
		6.82	1	82		64			29	23		64	60		73	50
6-64	OLd	6				4.79			25.9	25		64			36	
		6.53		1		4	79		25.2			64			72	
						4	79		27.9			64			72	99
12-64	re	30	1	30		88		62	22	26		64			64	
		3	1	84		78		82	24.9	23		64			71	
				79								64			71	
8-64	Ste	21	6	30		62	72	62	20.3	26		64	12		74	00
		6				72	69	72	22	27		64	14			
						72	4.82	27	21			70	6.3			
24-64	re II	44	2.3	64		70	82		24	24		64			74	
				30	4	72		10	30			64			30	74
24-64	pool II	7.60		60	2	20	75	62	28			62			70	
		6		78	-0.2	0	70	61	21	27						
					-0.6	0	70		24			74	64	14		77
21-64	pool II	62		47		62		62		26		64			72	00
		36		29	3	64	72	62	24	24		64			69	64
		90	2.3	80	1	1		82	21	22		64			74	74
2-1-62	pool III	96		0		3	67	23				64	70		75	70
		7.62		60		44	60	50	54	23		64			70	00

P 1 1 1 1 1 1 1

6-164	m	made	67	myeloma	(f type)	60	73		24		61	64				
			67	60			73		24							
			52	69	3.2		75		24							
			30	30					24							
26-164	VO	male	73	myeloma	(f type)	60	88	62	27		81.2			74	25	
		7	73	60			88	64	23							
			96	60				64			64					
2-1-64	re II	male	77	myeloma	(f type)	60	71	88	24		81	85		64		
			77	60	3		71	88	24		81	85		64		
			94	60			71	88	24		81	85		64		
21-1-64	re II	64		2	60	66	60		26		65	72		64		
		32		29	60	66	64	72	26		64	74		64		
		94		60	4.2	66	64	27			64			64		
27-34	La	female	60	myeloma	(f type)	60	60		24		74	67		72		
		74		72	1	64			24		64					
						64	60	26			64					

b      ti      i      pH      g      7 10      7 50  
(m      III      )

pH	<u>Na</u>		<u>K</u>		<u>Ca Mg</u>	
	dial. sol.	$\Delta$	dial. sol.	$\Delta$	dial. sol.	$\Delta$
	mEq per Kg $H_2O$	mEq per 100 g prot	mEq per Kg $H_2O$	mEq per 100 g prot	mEq per Kg $H_2O$	mEq per 100 g prot
mean 14 7.27	1.20	2.0	4.34	0.71	4.06	2.44
Na and K over-acted for Lime photometer error:	14.19	2.7	4.34	0.90		

I      II  
Ca Mg dialysis solution      Ca+Mg dialysis solution  
mean 4.50 mEq/Kg  $H_2O$       mean 4.00 mEq/Kg  $H_2O$   
range 4.00 to 4.25      range 3.47 to 4.52

pH range	pH mean	$\Delta$ Ca Mg		pH mean	$\Delta$ Ca+Mg	
		mean	mEq per 100 g prot		mean	mEq per 100 g prot
6.20 to 6.75	6	2.90	2.06	6	2.56	2.01
to 6.9	6	6.23	2.23	6	6.25	2.23
7.00 to 7.25	6	7.1	2.01	6	7.11	2.51
7.5 to 8.0	6	7.20	2.1	10	7.20	2.15
7.50 to 8.00	6	7.64	0.5	9	7.60	4.52



b 11 1 PH 7 10-7 30

		pH	MEQ
	number	mean value	mean value
	observed		mEq per 100 g prot
plasma	3	7.39	12.9
serum	14	7.33	16.4
	correction flame photomet	res	0.3
	correction for albumin-protein		0.3
serum corrected	14	7.33	17.0

mean protein concentration 0.23 g/Kg H<sub>2</sub>O

dal	serum	pH range		regression line
				NCE on pH
3-4.81	pool I	8.35 to 7.60	3	8.36 (pH 5.56)
18-3.61	pool II	8.78 to 7.37	3	11.06 (pH 5.53)
21-3.61	pool III	8.65 to 7.39	3	8.88 (pH 5.77)
3-1.82	pool IV	8.99 to 7.98	3	5.39 (pH 6.41)
19-4.81	Le (S)	8.69 to 7.59	3	7.37 (pH 6.20)
3-5.61	Dra	8.65 to 7.42	3	11.95 (pH 6.01)
8-5.61	Le (M)	8.78 to 7.28	3	12.49 (pH 6.28)
14-5.61	Le Le	8.65 to 7.34	3	10.68 (pH 5.83)
18-5.61	dV	8.96 to 7.70	3	11.55 (pH 6.06)
24-5.61	vdW	7.09 to 7.74	3	7.25 (pH 6.97)
29-5.61	dCe	7.14 to 7.63	3	17.26 (pH 6.42)
1-6.41	dH	8.82 to 7.46	3	8.78 (pH 6.72)
8-6.41	dLe	8.33 to 7.15	3	8.23 (pH 6.02)
13-6.41	Vre	8.82 to 7.37	3	8.39 (pH 6.73)
19-6.41	Bir	8.78 to 7.28	3	12.16 (pH 5.74)
	g	g	1 11	10.01 (pH 6.70)

Note- Indicate the number of point from which the regression line was calculated.

pH	I		pH		II		pH	
	(mM)		(mM)		(mM)		(mM)	
	Ca		Mg		Ca		Mg	
	dial. sol.	$\Delta$	dial. sol.	$\Delta$	dial. sol.	$\Delta$	dial. sol.	$\Delta$
	mEq per Kg $H_2O$	mEq per 100 g prot	mEq per Kg $H_2O$	mEq per 100 g prot	mEq per Kg $H_2O$	mEq per 100 g prot	mEq per Kg $H_2O$	mEq per 100 g prot
mean	142.9	8	434	0.31	496	2.64		
and X corrected for some photometer error:	141.9	8.2	434	0.35				

pH range	I			II		
	Ca+Mg dialysis solution			Ca+Mg dialysis solution		
	mean	4.83 mEq/Kg $H_2O$		mean	96 mEq/Kg $H_2O$	
	range	4.69 to 4.97		range	74 to 4	
	pH	$\Delta$ Ca Mg		pH	$\Delta$ Ca+Mg	
	mean	mean		mean	mean	
		mEq per 100 g prot			mEq per 100 g prot	
6.50 to 6.75	4	0.33	2.90	8.96	2.83	
6.75 to 7.00	8	0.52	2.32	9	0.83	2.25
7.00 to 7.25	7.1	2.3		8	2.11	2.2
7.5 to 8.0	10	30	2.41	10	2.38	2.44
7.50 to 8.00	6	2.54	4.03	9	2.60	4.34

sample number	pH (lithide) range	mean	ion	concentration dialysed sol. [C] [m]	mEq/Kg H <sub>2</sub> O	t		t.t	(P <sub>calc</sub> K)
						distilled water			
						mean	S.E.		
31	7.20 to 7.75	7.48	Na	142.2	1.0001	0.0008	0.9453	0.0017	
			K	4.21	0.9999	0.0031	0.9610	0.0048	
			C Mg	4.01	0.9999	0.0016	0.9610	0.0096	
			Cl	120.8	0.9994	0.0014	0.9782	0.0020	
			HCO <sub>3</sub>	23.9	1.0021	0.0055	0.9812	0.0038	
31	8.00 to 7.90	8.01	N	142.8	1.0001	0.0003	0.9848	0.0022	
			K	4.48	0.9992	0.0019	0.9706	0.0044	
			C Mg	4.11	1.0014	0.0011	0.9727	0.0077	
			Cl	126.4	1.0002	0.0008	0.9928	0.0032	
			HCO <sub>3</sub>	23.7	0.9999	0.0041	0.9811	0.0039	

Mod. all serum data from table 47 re included except those where pH inside was not measured (3) and those where the pH was outside the range indicated above (2). The concentration ratio refers to protein concentration of 7.0 g/Kg H<sub>2</sub>O the actual protein concentration was on the average 8.28 g/Kg H<sub>2</sub>O. The observation in the high pH range and 8.04 g/Kg H<sub>2</sub>O for those in the low pH range. No correction has been made for systematic error.

pH range	ion	R <sub>cat</sub>	
		uncorrected	corrected for Dumas photometer error
2.30 to 2.75	Na	0.9563	0.9597
	K	0.961	0.94
6.50 to 7.50	Na	0.9645	0.9641
	K	0.971	0.95

plasma	l i g h t		p i m		l w h i h b u		R <sub>Ca</sub> (prot 70 g / Kg H <sub>2</sub> O)	R <sub>Mg</sub>		
	C		d C M g		d m i d					
	pH inside	dialysis solution	Ca	Mg	ΔCa	ΔMg				
		mEq/Kg H <sub>2</sub> O			mEq/100 g prot					
Ca <sub>i</sub>	7.48	2.02	1.20		2.81	0.87	0.502	0.553		
Yre (S)	20	2.75	25		2.94	0.96	0.806	0.542		
vdP	34	3.4	1.5		2.33	0	0.542	0.55		
vdB	29*	2.60	1.75		2.94	1.03	0.566	0.615		
mean values	45	2.80	1.75		2.90	1.00	0.5690	0.6040		
Note pH dialysis solution										











TABLE I															
W. CH.		14		1		L. OF T.		W.		number data and		W. CH.		Δ	
Rate and protein preparations		at rate 17-18°C		at rate 18-19°C		at rate 20-21°C		at rate 22-23°C		at rate 24-25°C		at rate 26-27°C		at rate 28-29°C	
		pH		pH		pH		pH		pH		pH		pH	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	
		mM		mM		mM		mM		mM		mM		mM	

# COMBINATION RATIO PROTEIN CONCENTRATION W / R<sub>0</sub> #1

100%  
 100% 100% 100%  
 100% 100% 100%

100% 100% 100%  
 100% 100% 100%  
 100% 100% 100%

100% 100% 100%  
 100% 100% 100%  
 100% 100% 100%

100% 100% 100%

100% 100% 100%  
 100% 100% 100%  
 100% 100% 100%

100% 100% 100%  
 100% 100% 100%  
 100% 100% 100%

100%

100%

100%

100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%  
 100% 100% 100% 100% 100%  
 100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

100% 100% 100% 100% 100%

(56) 175

date experiment	pH inside	alb		100 lectr
		alb	glob	
		M	hour	
16-560	7.27	41.7		
18-560	7.39	38.0		
21-562	7.32	48.6	48.5	
26-562	8.03	49.3	48.5	
25-642	7.70		48.5	
	7.00		47.4	
	6.67		46.3	

(57) 178

	pH inside	NCE m E
<u>albumin</u>	7.44 (8)	12.7 (8)
<u><math>\gamma</math>-globulin</u>	7.47 (4)	2.3 (4)

(normal serum pH 7.45 17.5

Not the number of experiments from  
indicated between brackets  
these are discussed in the text

(58) 179

pH range		ion		pH inside
7.20	7.70	M Cl	8	7.44
6.60 to 7.20		Na Cl	7	6.80

Not the values are mean values and have  
Correction of  $N_M$  for the (approximate)  
the values in the case of  $\gamma$ -globulin, but  
at pH 7.44 0.942 0.944  
at pH 6.80 0.933 0.937

The ion concentrations in the dialysis solution  
with albumin since these will be needed in it

	<u>in vivo ultrafiltration</u> (plasma)	<u>equilibrium dialysis</u> (serum)
number of experiments	22	4
number of subjects	22	13
pH range	7.2 to 7.39	7.14 to 7.42
<u>pH mean value</u> <u>corrected for</u> <u>systematic error</u>	7.24	7.2
<u>PCE mean value (mEq/100 g prot)</u> <u>corrected for</u> <u>systematic error</u>	17.5	17.0

	A		B		A minus B
	<u>in vivo ultrafiltration</u> (plasma)		<u>equilibrium dialysis</u> (serum)		difference in BCE after adjusting PCE of B to pH of A
	pH	BCE mEq per 100 g prot	pH	BCE mEq per 100 g prot	
Obs	7.24	2	7.44	18.1	1.7
Obs	7.18	1	7.43	18.1	-2.4
Lo	7.22	0.5	7.4	17.4	1.5
dy	7	4.7	7.15	16.6	2.5
dCo	7.5	0	7.4	17.5	4.5
Lo	7.1	0.5	7.4	17.5	1.5
Lo	7.2	0	7.5	1.5	-0.5
Lo	7.2	1.4	7.7	6	3.6
B	7.2	1.1	7.2	1	-0.4
mean value	7		7.2		

correction for

Blank photometer  
error

equilibrium protein

temperature blank

the  
in vivo ultrafiltration

Note the steps listed for BCE made in vivo ultrafiltration in Study  
showed the error in the error due to pH change ring  
adjustment and for error caused by the addition of heparin

	pH		Na		Ca Mg	
	mean		'free' mEq per Kg H <sub>2</sub> O	'bound' mEq per 100 g prot	'free' mEq per Kg H <sub>2</sub> O	'bound' mEq per 100 g prot
<u>in vivo ultrafiltration</u> <u>and corrected for</u> <u>Duncan equilibrium</u>	7.2	34	28.2	1	4.02	2.79
<u>equilibrium dialysis</u> <u>and corrected for</u> <u>Duncan equilibrium</u>		7.22	42.8	3.2	4.06	2.44
<u>corrected for</u> <u>Duncan equilibrium</u>	1	7.22	136.2	36.3	2.00	2.03

Table 1 of 1b m i d f g i b i i

dal experiment	pH inside	alb		NCE		$\Delta N$		$\Delta Ca$ Mg	
		alb	glob	mEq/100 g p		t i		t i	
		II joor	lectr	alc	found	calc	found	alc	found
16-540	7.37	41.7		7.3	7.7	4.9	8.1	2.57	2.27
21-840	7.39	41.6		10.1	10.5	10.8	11.0	2.96	2.22
22-342	7.32	42.6	41.5	7.2	6.9	8.5	4.9	2.01	1.96
24-143	6.83	42.2	42.8	3.4	3.8	4.4	4.3	0.84	1.00
25-1042	7.70		42.3	7.3	8.7	6.3	6.6	3.22	2.31
	7.00		47.4	3.7	4.2	3.8	3.6	1.76	1.92
	6.81		42.2	2.5	2.6	2.3	2.6	1.29	1.22

Table 1 of 1b m i d f g i b i i

	pH inside	NCE		$\Delta Na$		$\Delta K$		$\Delta Ca$ Mg	
		mEq p		100 g p		t i		t i	
<u>Bumj</u>	7.44 (8)	10.7 (8)	11.3 (8)	0.25 (8)	4.60 (7)				
<u><math>\gamma</math> globulin</u>	7.47 (4)	11.2 (4)	11.1 (4)	0.09 (4)	1.03 (3)				
(normal serum t pH 7.45 11.5 8.5 0.10 2.15)									

Not the number of experiment from which the mean was calculated (indicated between brackets). No correction was made for systematic errors (these are discussed in the text).

Table 1 of 1b m i d f g i b i i

pH range	ion	pH inside	concentr dial sol. mEq po Kg H <sub>2</sub> O	$R_{cat}$ $R_{an}$	pH inside	$R_{cat}$ $R_{an}$
7.20-7.70	Na	7.44	132.5	0.943	7.47	1.002
	Cl		118.2	0.981		1.010
6.60 to 7.20	Na	6.80	142.2	0.928	6.81	1.005
	Cl		130.3	0.959		1.011

Not the value are mean values and have not been corrected for systematic error. Correction of  $R_{Na}$  for the (approximate) flame photometer error would not alter the values in the case of  $\gamma$  globulin, but for Bumj  $R_{Na}$  would become at pH 7.44 0.942 0.944 (see discussion in the text) at pH 6.80 0.935 0.957.

The ion concentrations in the dialysis solution are only given in the appendix (see with albumin since these will be needed in chapter VIII).





# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 421

## SPECIFIC AND NONSPECIFIC IMMUNITY IN *CANDIDA* INFECTIONS

Experimental Studies of the Role of *Candida* Cell Constituents  
and Review of Literature

By

BOHDAN DOBIAŁ

ACCOMPANIES VOL. 176

---

STOCKHOLM 1964



**AMSTERDAM 1964**  
**SCHELTEMA & HOLKEMA N.V**

Department of Dermatology College of Physicians and Surgeons, Columbia University,  
New York, New York

# SPECIFIC AND NONSPECIFIC IMMUNITY IN *CANDIDA* INFECTIONS

Experimental Studies of the Role of *Candida* Cell Constituents  
and Review of Literature

By

BOHDAN DOWIAŁ, M. D.

STOCKHOLM 1964

# ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of *Nordiskt Medicinskt Arkiv* founded in 1869 by Axel Key. The first volume of *Acta Medica Scandinavica* is therefore numbered LII (52).

The chief editors have been: Axel Key 1869 — 1900, C. G. Santesson 1901 — 1915, I. Holmgren 1916 — 1957 and Birger Brändell 1958 to date.

*Acta Medica Scandinavica* publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board. Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the Editor at the address below.

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that, if accepted, it will not be republished in any other journal in the same or similar form, without the Editor's written consent.

Papers will be published in English, French or German at the authors' choice, followed by a summary in English.

The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left-hand margin.

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author. No paper shall exceed 24 printed pages.

## Subscription

The annual subscription to the journal, covering two volumes, each of 6 numbers is 140 Sw. crowns or U. S. \$ 27.25 including postage in the Scandinavian countries and in Holland 120 Sw. crowns.

*Address for subscriptions and all communications*

ACTA MEDICA SCANDINAVICA

P. O. Box 2052 Stockholm 2

---

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

*Department of Dermatology College of Physicians and Surgeons, Columbia University*

*New York, New York*

# **SPECIFIC AND NONSPECIFIC IMMUNITY IN CANDIDA INFECTIONS**

**Experimental Studies of the Role of *Candida* Cell Constituents  
and Review of Literature**

By

**BONDAN DOBIEZ, M. D.**

**STOCKHOLM 1964**

# ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of *Nordiskt Medicinskt Arkiv* founded in 1869 by Axel Key. The first volume of *Acta Medica Scandinavica* is therefore numbered LII (52).

The chief editors have been. Axel Key 1869 — 1900, C. G. Santesson 1901 — 1915, I. Holmgren 1916 — 1957 and Burger Brändell 1958 to date.

*Acta Medica Scandinavica* publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board. Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the Editor at the address below.

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that, if accepted, it will not be republished in any other journal in the same or similar form without the Editor's written consent.

Papers will be published in English, French or German at the authors' choice, followed by a summary in English.

The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left-hand margin.

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author. No paper shall exceed 24 printed pages.

## Subscription

The annual subscription to the journal covering two volumes, each of 6 numbers is 140 Sw. crowns or U. S. \$ 27.25 including postage in the Scandinavian countries and in Holland 120 Sw. crowns.

*Address for subscriptions and all communications*

ACTA MEDICA SCANDINAVICA

P. O. Box 2052 Stockholm 2

---

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

# TABLE OF CONTENTS

(1) INTRODUCTION	5
(2) EXPERIMENTAL	
(a) Materials and Methods	7
(b) Specific Immunization with Cell Walls of <i>Candida albicans</i>	8
(c) Failure to Confer Specific Immunity with Soluble Extract of <i>Candida albicans</i>	8
(d) Changes in Nonspecific Resistance to <i>Candida albicans</i> Induced by Soluble Extract of <i>Candida albicans</i> and Endotoxin of <i>Escherichia coli</i>	9
(e) Changes in Nonspecific Resistance to <i>Salmonella enteritidis</i> Induced by Soluble Extract of <i>Candida albicans</i> and Endotoxin of <i>Escherichia coli</i>	12
(f) Sensitization with Soluble Extract of <i>Candida albicans</i>	13
(3) REVIEW OF LITERATURE	15
(A) Nonspecific Host Resistance	16
(i) ( ) Effect of Dead Organisms, their Products or Substances not Derived from Microorganisms on Nonspecific Resistance to <i>Candida</i>	17
(i) (b) Effect of Dead <i>Candida</i> or its Products on Nonspecific Resistance to <i>Candida</i> Infections	20
(i) (c) Effect of Dead <i>Candida</i> or its Products on Resistance to Other Microorganisms	21
(ii) (a) Effect of Double Infections with <i>Candida</i> and Another Organism on Nonspecific Resistance	24
(ii) (b) Effects of Living <i>Candida</i> on Nonspecific Resistance to a <i>Candida</i> Infection	28
(B) Allergy and its Relation to Immunity in Moniliasis	31
(i) Attempts to Produce Bronchopulmonary Moniliasis without Sensitization	31
ii) Production of Active and Passive Anaphylaxis with <i>Candida</i> or its Products	31
) Production of Bronchopulmonary Moniliasis in Sensitized Animals	33
(C) Specific Immunity	
(i) Active Immunization	35
(a) Active Immunization with Living Cells in Rabbits	35

Printed in Sweden  
LUNGL. BOKTRYCKERIET P. A. NORSTEDT & SÖNER  
STOCKHOLM 1961

## (1) INTRODUCTION

In spite of recent progress in the treatment of moniliasis it has become apparent that cases of the disseminated form of this disease are difficult to handle with the present therapeutic methods, including antifungal antibiotics (103). The incidence of generalized moniliasis may not be entirely negligible (17, 72, 144) and epidemics of moniliasis may occasionally occur particularly among institutionalized young children (14, 96, 103, 142). The role of antibacterial antibiotics in the enhancement of the disease has often been stressed but never satisfactorily explained (30). The possible role of nonspecific resistance in this phenomenon has not been explored. For these reasons it appears worth while to examine the role of immunity in the pathogenicity of *Candida* in general and to investigate the applicability of immunologic methods for the protection against moniliasis and for the treatment of disseminated forms of the disease.

Although a voluminous serologic literature deals with taxonomic considerations and diagnostic tests (63, 110, 125, 131, 132) comparatively little experimental work has been devoted to the study of immunization against moniliasis.

The reason may be that since natural immunity may not be clearly demonstrated following monilial infection a development of an anti-*Candida* vaccine may not have been considered promising. It has been known that high agglutinin

and complement fixing titers often develop in the course of natural *Candida* infections (63, 104, 125, 131, 137) and following immunization (53, 104, 130, 137) but the reasons for the frequent lack of protection against challenge in such situations have remained obscure. The possible adverse effects of the endotoxin-like substance of *Candida* and of hypersensitivity on resistance to *Candida* infections has not been adequately studied. Elimination of the endotoxin-like substance and of sensitizing substances from experimental vaccines has not been tried.

A study of the relationship of the structure of *Candida* to its immunological properties may lead to a separation of an effective protective antigen from cell fractions with other functions. Although electron microscopic studies of *Candida* as well as some chemical studies of its cell constituents are available, only limited attempts at correlation of the many structural and chemical elements of the fungus have been made. Possible physiologic functions of the anatomical and chemical subcellular components have remained all but obscure.

Data from such studies may not only be applicable to *Candida* but they may be useful for the understanding of similar problems in other fungal organisms with a similar anatomic structure in the expectation that similar principles may be used for immunization against these



(b) Active Immunization with Living Cells in Guinea Pigs	37
(c) Active Immunization with Living Cells in Mice	38
(d) Active Immunization with Dead Cells in Rabbits	40
(e) Active Immunization with Dead Cells in Guinea Pigs	41
(f) Active Immunization with Dead Cells in Mice	41
(g) Active Immunization with Fractions or Products of <i>Candida</i> Cells	42
(h) Active Immunization of Animals Already Infected with <i>Candida</i>	42
(i) Passive Immunization	43
(D) Immunization in Humans	45
(i) Active Immunization	45
(ii) Passive Immunization	47
(4) DISCUSSION	
(a) Differences between Natural and Experimental Systemic Moniliasis	48
(b) Factors Influencing the Outcome of Specific Immunization	48
(c) Characteristics of the Soluble Extract of <i>Candida albicans</i>	53
(d) Characteristics of Cell Walls	54
(e) Role of Endotoxins in Nonspecific Resistance	57
(f) Possible Role of Bacterial Resistance-Influencing Substances in Pathogenesis of Moniliasis	58
(g) Possible Role of Resistance Influencing Substance of <i>Candida</i> in Pathogenesis of Moniliasis	61
(h) Applicability of Immunological Findings in Moniliasis to Other Fungus Diseases	62
(i) Possible Application of the Reported Findings in Therapy and Prevention of Systemic Mycoses	65
(j) Possible Bearing of the Reported Findings on the Pathogenesis and Management of Bacterial Infections Arising During Antibiotic Treatment	66
(5) SUMMARY	67
(6) LITERATURE CITED	72

## (1) INTRODUCTION

In spite of recent progress in the treatment of moniliasis it has become apparent that cases of the disseminated form of this disease are difficult to handle with the present therapeutic methods, including antifungal antibiotics (105). The incidence of generalized moniliasis may not be entirely negligible (17, 72, 144) and epidemics of moniliasis may occasionally occur particularly among institutionalized young children (14, 26, 103, 142). The role of antibacterial antibiotics in the enhancement of the disease has often been stressed but never satisfactorily explained (30). The possible role of nonspecific resistance in this phenomenon has not been explored. For these reasons it appears worth while to examine the role of immunity in the pathogenicity of *Candida* in general and to investigate the applicability of immunologic methods for the protection against moniliasis and for the treatment of disseminated forms of the disease.

Although a voluminous serologic literature deals with taxonomic considerations and diagnostic tests (63, 110, 125, 131, 152) comparatively little experimental work has been devoted to the study of immunization against moniliasis. The reason may be that more natural immunity may not be clearly demonstrated following monilial infection a development of an anti-*Candida* vaccine may not have been considered promising. It has been known that high agglutinin

and complement fixing titers often develop in the course of natural *Candida* infections (63, 104, 125, 131, 157) and following immunisation (53, 104, 150, 157) but the reasons for the frequent lack of protection against challenge in such situations have remained obscure. The possible adverse effects of the endotoxin-like substance of *Candida* and of hypersensitivity on resistance to *Candida* infections have not been adequately studied. Elimination of the endotoxin-like substance and of sensitizing substances from experimental vaccines has not been tried.

A study of the relationship of the structure of *Candida* to its immunological properties may lead to a separation of an effective protective antigen from cell fractions with other functions. Although electron microscopic studies of *Candida* as well as some chemical studies of its cell constituents are available, only limited attempts at correlation of the many structural and chemical elements of the fungus have been made. Possible physiologic functions of the anatomical and chemical subcellular components have remained all but obscure.

Data from such studies may not only be applicable to *Candida* but they may be useful for the understanding of similar problems in other fungal organisms with a similar anatomic structure in the expectation that similar principles may be used for immunization against these

(b) Active Immunization with Living Cells in Guinea Pigs	37
(c) Active Immunization with Living Cells in Mice	38
(d) Active Immunization with Dead Cells in Rabbits	40
(e) Active Immunization with Dead Cells in Guinea Pigs	41
(f) Active Immunization with Dead Cells in Mice	41
(g) Active Immunization with Fractions or Products of <i>Candida</i> Cells	42
(h) Active Immunization of Animals Already Infected with <i>Candida</i>	42
(ii) Passive Immunization	43
(D) Immunization in Humans	45
(i) Active Immunization	45
(ii) Passive Immunization	47
 (4) DISCUSSION	
(a) Differences between Natural and Experimental Systemic Moniliasis	48
(b) Factors Influencing the Outcome of Specific Immunization	48
(c) Characteristics of the Soluble Extract of <i>Candida albicans</i>	53
(d) Characteristics of Cell Walls	54
(e) Role of Endotoxins in Nonspecific Resistance	57
(f) Possible Role of Bacterial Resistance-Influencing Substances in Pathogenesis of Moniliasis	58
(g) Possible Role of Resistance Influencing Substance of <i>Candida</i> in Pathogenesis of Moniliasis	61
(h) Applicability of Immunological Findings in Moniliasis to Other Fungus Diseases	62
(i) Possible Application of the Reported Findings in Therapy and Prevention of Systemic Mycoses	65
(j) Possible Bearing of the Reported Findings on the Pathogenesis and Management of Bacterial Infections Arising During Antibiotic Treatment	66
 (5) SUMMARY	67
 (6) LITERATURE CITED	72

## (1) INTRODUCTION

In spite of recent progress in the treatment of moniliasis it has become apparent that cases of the disseminated form of this disease are difficult to handle with the present therapeutic methods, including antifungal antibiotics (105). The incidence of generalized moniliasis may not be entirely negligible (17, 72, 144) and epidemics of moniliasis may occasionally occur particularly among institutionalized young children (14, 26, 103, 142). The role of antibacterial antibiotics in the enhancement of the disease has often been stressed but never satisfactorily explained (30). The possible role of nonspecific resistance in this phenomenon has not been explored. For these reasons it appears worth while to examine the role of immunity in the pathogenicity of *Candida* in general and to investigate the applicability of immunologic methods for the protection against moniliasis and for the treatment of disseminated forms of the disease.

Although a numerous serologic literature deals with taxonomic considerations and diagnostic tests (63, 110, 115, 131, 132) comparatively little experimental work has been devoted to the study of immunization against moniliasis. The reason may be that since natural immunity may not be clearly demonstrated following monilial infection development of an anti-*Candida* vaccine may have been considered promising. It has been known that high agglutination

and complement fixing titres often develop in the course of natural *Candida* infections (63, 104, 125, 131, 137) and following immunization (53, 104, 130, 137) but the reasons for the frequent lack of protection against challenge in such situations have remained obscure. The possible adverse effects of the endotoxin-like substance of *Candida* and of hypersensitivity on resistance to *Candida* infections have not been adequately studied. Elimination of the endotoxin-like substance and of sensitizing substances from experimental vaccines has not been tried.

A study of the relationship of the structure of *Candida* to its immunological properties may lead to a separation of an effective protective antigen from cell fractions with other fractions. Although electron microscopic studies of *Candida* as well as some chemical studies of its cell constituents are available, only limited attempts at correlation of the many structural and chemical elements of the fungus have been made. Possible physiologic functions of the anatomical and chemical subcellular components have remained all but obscure.

Data from such studies may not only be applicable to *Candida* but they may be useful for the understanding of similar problems in other fungal organisms with a similar anatomic structure in the expectation that similar principles may be used for immunization against these

organisms and for the explanation of various aspects of their pathogenicity.

The purpose of this paper is to report experimental results concerning the effects of cell constituents of *Candida albicans* prepared by mechanical disintegration of its yeast cells, as far as they have relation to immunization against and hypersensitivity to the fungus. Their effect on nonspecific resistance against other organisms will also be reported. A review of literature was undertaken on specific and nonspecific immunity in experimental and human moniliasis, and on hypersensitivity in disseminated monial infection another factor which appears to influence the resistance to the disease.

The applicability of principles of nonspecific resistance for the explanation of the apparent enhancement of moniliasis and of bacterial infections not susceptible to antibiotics during antibiotic treatment and after the use of various vaccines will be discussed in view of the finding that endotoxins of gram-negative organisms enhance under certain conditions the growth of *Candida* and of other organ-

isms in vivo. Methods directed towards neutralizing endotoxins will be mentioned as possible means of counteracting the enhancement of fungal and bacterial infections by antibiotics and the constant stimulation of growth of fungi by endotoxin like substances which may take place in systemic mycoses. The possible use of active and passive specific immunization for the protection against and treatment of moniliasis will be discussed. The applicability of the findings concerning the immunological properties of various cell constituents of *Candida* to other fungi will also be discussed.

The report on own experimental findings is followed rather than preceded by a critical review of experiments of other workers concerning the relation of allergy to immunity in moniliasis, and those dealing with immunizations, since it is believed that experimental results reported here particularly those concerning nonspecific resistance and hypersensitivity are helpful in evaluating the mechanism of action of past experimental findings in a new light.

## (2) EXPERIMENTAL

### (a) Materials and Methods

**Microorganisms.** — Strain No. 17992h of *Candida albicans* was used in all experiments. It was originally isolated in 1955 from the blood stream of a patient with systemic moniliasis. For the preparation of intravenous inoculations the organism was grown on Sabouraud glucose agar in Petri dishes for 48 hours at the temperature of 37° C. Fresh cultures were prepared for each inoculation. The yeast cells were counted in hemocytometer. The same strain was grown for the production of a filtrate in Roux bottles on Sabouraud glucose agar at room temperature for 3 days.

*Salmonella enteritidis* strain B 27 was kindly provided by Dr. C. A. Slanetz from the Department of Aerial Care, College of Physicians and Surgeons. This strain was originally isolated from rabbit. A fresh culture was grown for 24 hours at 37° C. prior to each challenge. The organism was transferred each time by placing 0.2 cc. of culture material in 10 cc. of nutrient broth.

**Extract from *Microgaster*.** — The reduction of *Escherichia coli* was a lipopolysaccharide of E. coli O26 B6 obtained from the DuPont Laboratories, Detroit. It was prepared by the Borovitch chloroacetic acid procedure as modified by Webster, Sagan, Lanch and Johnson (1946).

**Cell fractions of *Candida albicans*.** — Cells were prepared from the fungus grown as described. The growth occurred almost exclusively of cast cells. The harvested cells were subsequently washed 5 times in water. They were then mechanically disrupted in a homogenizer described by Vertesschlager, Schloeman and Kurz (1961) and manufactured by B. Braun, Melsungen, Germany. Tissue extracts of 50% suspension

of *Candida* cells in distilled water were added to 50 gm. of glass beads (Ballotini No. 31/3, diameter 0.5 mm., manufactured by Dragon-Werk Georg Wild, Bayreuth, Germany) in a bottle with a capacity of 75 cc. The chamber in which this bottle was rotating eccentrically was cooled with liquid carbon dioxide. After a treatment lasting about 8–9 minutes an almost complete disintegration of the yeast cells was observed under the microscope where cell fragments of various sizes were seen. Counts on pour plates done on 2 batches of cell walls revealed that one of about 100,000 cells was viable. The cell debris was then shaken for an hour and a half on aibrator (Synatron Paper Jagger Type PJ 4). The suspension was kept in refrigerator overnight. The next day it was shaken again for 1 hour. It was then centrifuged at 14,500 RPM (about 27,000 G) for 4 hours. The supernatant was filtered through an ultra-fine ground glass filter. The filtrate which was clear straw-colored fluid was homogenized, stored in refrigerator and reconstituted with physiologic saline solution just before use.

The sediment was washed 5 times with water homogenized, stored for future use in refrigerator or reconstituted at least 24 hours before use in physiologic saline solution containing 0.01% Merthiolate.

One mg. of cell walls was prepared from approximately 250,000,000 cells, one mg. of the soluble extract from about 100,000,000 cells.

**Animal.** — Male hot Swiss mice weighing 20–25 gm. were housed 10 to a metal cage fed commercial pellets (supplied by Hemlock Hollow Farm, Wayne, N. J.) and given water *ad libitum*. Male guinea pigs weighing 180–220 gm. were used in hyperimmune experiments.

organisms and for the explanation of various aspects of their pathogenicity.

The purpose of this paper is to report experimental results concerning the effects of cell constituents of *Candida albicans* prepared by mechanical disintegration of its yeast cells, as far as they have relation to immunization against and hypersensitivity to the fungus. Their effect on nonspecific resistance against other organisms will also be reported. A review of literature was undertaken on specific and nonspecific immunity in experimental and human moniliasis, and on hypersensitivity in disseminated monilial infection, another factor which appears to influence the resistance to the disease.

The applicability of principles of non-specific resistance for the explanation of the apparent enhancement of moniliasis and of bacterial infections not susceptible to antibiotics during antibiotic treatment and after the use of various vaccines will be discussed in view of the finding that endotoxins of gram-negative organisms enhance under certain conditions the growth of *Candida* and of other organ-

isms *in vivo*. Methods directed towards neutralizing endotoxins will be mentioned as possible means of counteracting the enhancement of fungal and bacterial infections by antibiotics and the constant stimulation of growth of fungi by endotoxin-like substances which may take place in systemic mycoses. The possible use of active and passive specific immunization for the protection against and treatment of moniliasis will be discussed. The applicability of the findings concerning the immunological properties of various cell constituents of *Candida* to other fungi will also be discussed.

The report on own experimental findings is followed rather than preceded by a critical review of experiments of other workers concerning the relation of allergy to immunity in moniliasis, and those dealing with immunizations, since it is believed that experimental results reported here particularly those concerning nonspecific resistance and hypersensitivity are helpful in evaluating the mechanism of action of past experimental findings in a new light.

## (2) EXPERIMENTAL

### (1) Materials and Methods

**Microorganisms.**— Strain No. 17992K of *Candida albicans* was used in all experiments. It was originally isolated in 1955 from the blood stream of patient with systemic moniliasis. For the preparation of intravenous inoculations the organism was grown on Sabouraud's glucose agar in Petri dishes for 48 hours at the temperature of 37 C. Fresh cultures are prepared for each inoculation. The yeast cells were counted in hemocytometer. The same strain was grown for the production of cell fractions in Roux bottles on Sabouraud's glucose agar at room temperature for 3 days.

*Salmonella enteritidis* strain II 270 was kindly provided by Dr. C. A. Slanetz from the Department of Animal Care, College of Physicians and Surgeons. This strain was originally isolated from rabbit. A fresh culture was grown for 24 hours at 37 C prior to each challenge. The organisms were transferred each time by placing 0.2 cc. of culture material in 10 cc. of nutrient broth.

**Extract from Microorganisms.**— The endotoxin of *Escherichia coli* was lipopolysaccharide of *E. coli* 026 B6 obtained from the Difco Laboratories, Detroit. It was prepared by the Borovitchloroacetic acid procedure as modified by Webster, Sagin, Landy and Johnson (146).

Cell fractions of *Candida albicans* were prepared from the fungus grown as described. The growth consisted almost exclusively of yeast cells. The harvested cells were subsequently washed 3 times with water. They were then mechanically disrupted in cell homogenizer described by Merckenschlager, Schloemann and Kurz (86) and manufactured by B. Brann, Melsungen, Germany. Twenty cc. of 50% suspension

of *Candida* cells in distilled water were added to 50 gm. of glass beads (Ballochini No. 31/B, diameter 0.5 mm., manufactured by Dragon-Werk Georg Wild, Bayreuth, Germany) in bottle with capacity of 75 cc. The chamber in which this bottle was rotating eccentrically was cooled with liquid carbon dioxide. After a treatment lasting about 8–9 minutes an almost complete disintegration of the yeast cells was observed under the microscope where cell fragments of various sizes were seen. Counts on pour plates done on 2 batches of cell walls revealed that one of about 100,000 cells was viable. The cell debris was then shaken for an hour and half on a vibrator (Syntro Paper Jigger Type PJ 4). The suspension was kept in refrigerator overnight. The next day it was shaken again for 1 hour. It was then centrifuged at 14,500 RPM (about 27,000  $\times g$ ) for 4 hours. The supernatant was filtered through an ultra-fine ground glass filter. The filtrate which was clear straw-colored fluid was lyophilized, stored in refrigerator and reconstituted with physiologic saline solution just before use.

The sediment was washed 3 times with water, lyophilized, stored for future use in refrigerator and reconstituted at least 24 hours before use in physiologic saline solution containing 0.01% Methylolane.

One mg. of cell walls was prepared from approximately 250,000,000 cells, one mg. of the soluble extract from about 100,000,000 cells.

**Animals.**— Male white Swiss mice weighing 20–25 gm. were housed 10 in metal cage, fed commercial pellets (supplied by Hemlock Hollow Farm, W. York, N. J.) and given water *ad libitum*. Male guinea pigs weighing 180–220 gm. were used in hypersensitivity experiments.



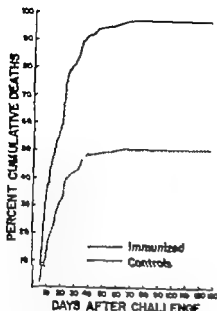


Fig 1 Cumulative death rates of mice immunized with cell walls of *Candida albicans* and challenged with 1,000,000 yeast cells of *C. albicans* intravenously (95 mice). Controls (100 mice) received 0.2 cc. of saline solution instead of the vaccine.

### (b) Specific Immunization with Cell Walls of *Candida albicans*

In preliminary experiments either 15, 9 or 6 injections of varying amounts of cell walls were administered subcutaneously to mice within 19 days. Eighteen days after the last injection mice were challenged intravenously with 1,000,000 cells of *Candida albicans*. The individual immunizing injections contained either 0.1 mg., 1 mg., 5 mg., 10 mg., 25 mg., 50 mg., 75 mg., 100 mg. or 200 mg. of cell wall material per injection. While no appreciable effect was noted with 0.1 mg. or 1 mg. the life of mice receiving 5 mg., 10 mg., 25 mg., 50 mg., 75 mg. or 100 mg. was considerably prolonged. Most mice receiving 200 mg. died in the course of immunization.

In another experiment 100 mice were injected 8 times with 5 mg. of the cell wall material in 0.2 cc. of physiological saline solution 38, 35, 33, 31, 28, 26, 24 and 21 days prior to intravenous challenge with 1,000,000 cells of *Candida albicans*. Five of the 100 mice died between the first immunizing in-

jection and challenge. The remaining 95 mice were observed daily for 126 days after challenge. The dead animals were autopsied. A control group of 100 mice received injections of 0.2 cc. of saline on the days on which other animals were immunized with the cell wall material. Ninety-seven of 100 control mice were dead at the end of the experiment while 48 (50.5 %) of 95 immunized animals died. Death rates in this experiment are illustrated in Figure 1.

On autopsies of both immunized and non-immunized animals lesions in various organs, mainly the kidneys, were noted. Lesions in most immunized animals appeared less extensive than those of the controls.

Ten mice similarly pretreated with cell walls of *Candida albicans* received an intraperitoneal injection of 0.2 cc. of a 1:20 dilution of a 24-hour broth culture of *Salmonella enteritidis* 21 days after the last vaccine injection. These mice died at about the same time as ten controls which received saline solution instead of the cell wall vaccine.

The mice given 5 mg. of cell wall material 8 times developed indurations at the sites of the subcutaneous injections most of which disappeared entirely after several weeks. Mice receiving higher doses of the cell walls occasionally developed necrotic lesions in the subcutaneous tissue at times with ulcerations of the skin.

### (c) Failure to Confer Specific Immunity with Soluble Extract of *Candida albicans*

Two experiments were performed to determine whether protection against a challenge with *Candida albicans* is possible by repeated subcutaneous injections of the soluble extract of the fungus the last one of which is given more than two weeks before challenge.

In one experiment total of 70 mice were given either 9, 10 or 32 subcutaneous injections of the soluble extract of *C. albicans* within 20 days. The doses ranged from 1 to 100 mg. of extract per injection. Fifteen days after the last injection the mice were challenged intravenously with either

1,200,000 or 12,000,000 cells. There was no difference between the survival times of immunized and nonimmunized mice.

In the second experiment a total of 80 mice were given either 9, 19 or 33 subcutaneous injections of the soluble extract of *Candida albicans* within 21 days. The doses again ranged from 1 to 100 mg. Eighteen days after the last injection the animals were challenged intravenously with 1,000,000 cells. There was no difference between the survival times of immunized and nonimmunized animals. Similarly as in the preceding experiment, mice receiving higher doses of the extract developed extensive alopecia in the course of the treatment.

(d) *Changes in Nonspecific Resistance to Candida albicans Induced by Soluble Extract of C. albicans and Endotoxin of Escherichia coli*

In an exploratory experiment 20 mice were given 33 intraperitoneal injections of 150 mg each of the soluble extract of *Candida albicans* within 21 days. Fourteen days later they were challenged intravenously with 6,000,000 living cells of *C. albicans*. Their survival time was 6 and 7 days, respectively as compared to 10 controls injected with saline in which the average survival time was 3.2 days.

This result pointed to the possibility that some protection could be afforded the animals when using the intraperitoneal route of inoculation and when shortening the time between the last injection and challenge. It appeared possible that the results of this experiment may be interpreted as an increase in nonspecific resistance to bacterial infections the effects of nonspecific resistance are of much more limited duration than those of specific immunity and intraperitoneal injections are much more effective in inducing it than subcutaneous ones.

My previous experiments showed that the soluble extract produced toxic symptoms and hematological changes similar to those caused by endotoxin of gram-negative bacteria. The experiment just described created suspicion that this endotoxin-like substance

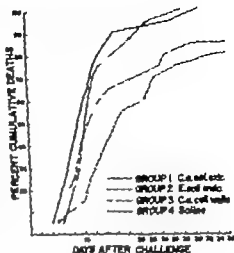


Fig. 2. Effect of an ip injection of 200 mg of soluble extract of *Candida albicans* (Group 1), 25 µg of endotoxin of *Escherichia coli* (Group 2) and 5 mg of cell walls of *C. albicans* (Group 3) on death rates of mice challenged iv with 6,000,000 yeast cells of *C. albicans* 5 days after pretreatment. Controls (Group 4) were pretreated with 0.5 cc. of saline solution ip. Each group 10 mice.

may share still another property with gram-negative endotoxins, namely their ability to modify nonspecific resistance to infections.

An experiment was set up, therefore, to test this possibility. Twenty mice were injected intraperitoneally with 200 mg of the soluble extract of *Candida albicans*. 20 mice received an intraperitoneal injection of 25 µg of the endotoxin of *Escherichia coli*. 20 animals were given 5 mg. of cell walls of *C. albicans* and 20 mice received 0.5 cc. of physiologic saline solution by the same route. Five days later all animals were given an intravenous injection of 6,000,000 viable yeast cells of *C. albicans*. Figure 2 shows that the survival times of mice pretreated with either the *Candida* extract or the endotoxin of *Escherichia coli* were prolonged. There was only a very slight prolongation of life in mice pretreated with cell walls of *C. albicans*.

In an experiment in which 20 mice were injected with 275 mg of the soluble extract

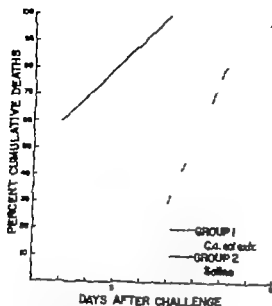


Fig 3 Effect of an ip injection of 275 mg of soluble extract of *Candida albicans* (Group 1) on death rates of mice challenged with 12,000,000 yeast cells of *C. albicans* 5 days later. Controls (Group 2) were pretreated with 0.5 cc. of saline solution ip. Each group, 20 mice.

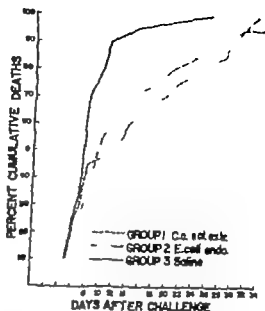


Fig 5 Effect of an ip injection of 200 mg of soluble extract of *Candida albicans* (Group 1), and of 35 µg of *Escherichia coli* endotoxin (Group 2) on death rates of mice challenged with 6,000,000 yeast cells of *C. albicans* 14 days later. Controls (Group 3) were pretreated with 0.5 cc of saline solution ip. Each group, 20 mice.

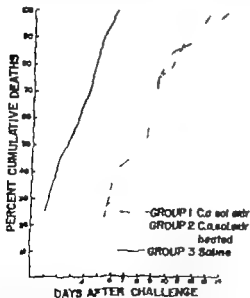


Fig 4 Effect of a ip injection of 275 mg of soluble extract of *Candida albicans* (Group 1) and of 275 mg of the same extract exposed to 60°C for 1 hour (Group 2) on death rates of mice challenged with 12,000,000 yeast cells of *C. albicans* 8 days later. Controls (Group 3) were pretreated with 0.5 cc of saline solution ip. Each group, 20 mice.

of *C. albicans* intraperitoneally 5 days before a challenge with 12,000,000 viable cells of *C. albicans* the survival time was considerably prolonged as compared with controls injected with saline instead of the extract (Figure 3).

A similar result was observed in mice in which the interval between the pretreatment and challenge was 8 days (Figure 4). In this experiment 20 mice were injected with 275 mg of the soluble extract of *C. albicans*, 20 mice with the same extract heated for 1 hour at 60°C and 20 mice with saline solution. There was no essential difference in mortality rates between mice treated with the heated and unheated extract. The effect of the *Candida* extract appeared later in mice challenged with 12,000,000 cells (Figure 3 and 4) than in those challenged with 6,000,000 cells (Figure 2). Figure 5 indicates that all of the control mice were dead 5 days after challenge, the day on which the first death occurred in the treated group. Some effect of both the *Can*

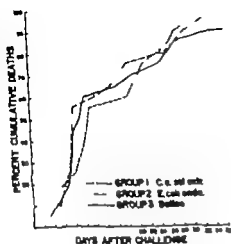


Fig. 6. Effect of an ip injection of 200 mg of soluble extract of *Candida albicans* (Group 1) and 35 µg of *Escherichia coli* endotoxin (Group 2) on death rates of mice challenged with 6,000,000 yeast cells of *C. albicans* 30 days later. Controls (Group 3) were pretreated with 0.5 cc. of saline solution ip. Each group 20 mice.

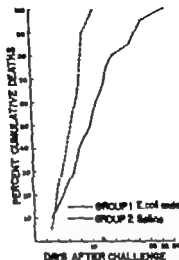


Fig. 8. Effect of an ip injection of 35 µg of *Escherichia coli* endotoxin (Group 1) administered 3 days after an iv injection of 6,000,000 yeast cells of *Candida albicans* on death rates of mice. Controls (Group 2) received 0.5 cc. of saline solution ip instead of endotoxin. Each group 20 mice.

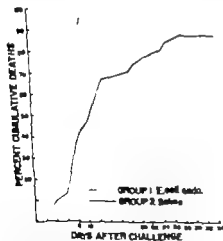


Fig. 7. Effect of an ip injection of 35 µg of *Escherichia coli* endotoxin (Group 1 20 mice) administered 3 hours after an iv injection of 6,000,000 yeast cells of *Candida albicans* on death rates of mice. Controls (Group 2 20 mice) received 0.5 cc. of saline solution ip instead of endotoxin.

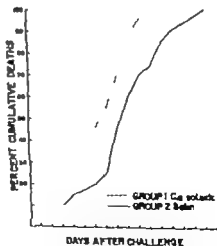


Fig. 9. Effect of an ip injection of 200 mg of soluble extract of *Candida albicans* (Group 1) administered 3 hours after an iv injection of 6,000,000 yeast cells of *C. albicans* on death rates of mice. Controls (Group 2) received 0.5 cc. of saline solution ip instead of the extract. Each group 20 mice.

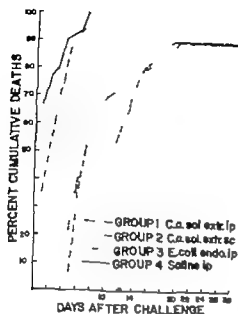


Fig 10 Effect of 2 ip (Group 1 20 mice) or sc (Group 2 20 mice) injections of soluble extract of *Candida albicans* given on first (150 mg.) and second (200 mg.) day of experiment, and of 2 ip injections of *Escherichia coli* endotoxin (Group 3 20 mice) given at the same times (25 µg. each time) on ip challenge with 0.15 cc of 1:10 dilution of a broth culture of *Salmonella enteritidis* given on the fourth day of the experiment. Controls (Group 4 30 mice) were pretreated with injections of 0.5 cc of saline solution ip.

*Candida* extract and *E. coli* endotoxin was still present when the interval between pretreatment and challenge was 14 days (Figure 5) but no effect on the survival times could be detected when the interval was 30 days (Figure 6).

The survival time of mice was shortened when *E. coli* endotoxin was injected 3 hours (Figure 7) or 3 days (Figure 8) after the *Candida* infection. A similar result was obtained when the soluble extract of *C. albicans* was injected 3 hours after the infection with *C. albicans* (Figure 9).

(c) *Changes in Nonspecific Resistance to Salmonella enteritidis Induced by Soluble Extract of Candida albicans and Endotoxin of Escherichia coli*

Twenty mice were injected intraperitoneally with 150 mg of the soluble extract of *C.*

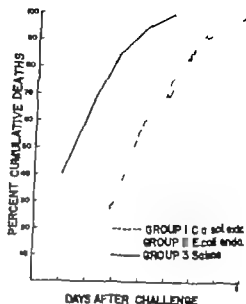


Fig 11 Effect of an ip injection of 200 mg soluble extract of *Candida albicans* (Group 1) and of 35 µg of *Escherichia coli* endotoxin (Group 2) on death rates of mice challenged with 0.2 cc. of a 1:20 dilution of a broth culture of *Salmonella enteritidis* 5 days after pretreatment. Controls (Group 3) were pretreated with 0.5 cc of saline solution ip. Each group, 20 mice.

*Candida albicans* on the first day of the experiment. On the second day 200 mg of the same material was injected intraperitoneally. Another group of 20 animals was injected at the same time subcutaneously with the same amount of the *Candida* extract. A third group of 20 animals received 2 injections of 25 µg each of the endotoxin of *E. coli* intraperitoneally on day 4. A fourth group of 30 animals served as controls and received physiological saline solution intraperitoneally. Mice in all 4 groups were challenged with an intraperitoneal injection of 0.2 cc of 1:20 dilution of a 24 hour broth culture of *Salmonella enteritidis* on the fourth day of the experiment. Animals pretreated intraperitoneally with the *Candida* extract or with *E. coli* endotoxin survived considerably longer than the controls which died prior to infection and two thirds of them (20 of 30) died within 4 hours. The last mouse of the control group died 7 days

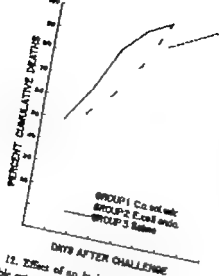


Fig. 12. Effect of an ip injection of 200 mg. of soluble extract of *Candida albicans* (Group 1) and of 33 µg. of endotoxin of *Escherichia coli* (Group 2) on death rates of mice challenged with 0.2 cc. of a 1:20 dilution of broth culture of *Salmonella enteritidis* 14 days after pretreatment. Controls (Group 3) were pretreated with 0.5 cc. of saline solution ip. Each group 20 mice.



Fig. 13. Effect of an ip injection of 200 mg. of soluble extract of *Candida albicans* (Group 1) and of 33 µg. of *Escherichia coli* endotoxin (Group 2) on death rates of mice challenged with 0.2 cc. of a 1:40 dilution of broth culture of *Salmonella enteritidis* 2 hours after the previous controls (Group 3) were pretreated with 0.5 cc. of saline solution ip. Each group 20 mice.

after inoculation. One third of mice per group were still alive after 13 days and one third of mice pretreated with *E. coli* endotoxin were alive 9 days after challenge. Only slight protection was observed among mice pretreated subcutaneously with the *Candida* extract. Figure 10 illustrates this experiment.

In another experiment (Figure 11) one group of 20 mice as given single intraperitoneal injection of 200 mg. of the soluble extract of *Candida albicans* the second group of 20 mice received single intraperitoneal injection of 33 µg. of the endotoxin of *E. coli* and the third group of 20 mice received saline solution. Five days after the pretreatment mice in all 3 groups were challenged with an intraperitoneal injection of 0.2 cc. of a 1:20 dilution of broth culture of *S. enteritidis*. Mice receiving *Candida* extract or *E. coli* endotoxin survived longer than the controls. Their sur-

vival time was not prolonged as much as that of mice which received 2 injections either the *Candida* extract or *E. coli* endotoxin in the preceding experiment. When the interval between pretreatment and challenge was 14 days the prolongation of life was only slight (Figure 12).

Enhancement of *Salmonella* infection was demonstrated when *Salmonella enteritidis* was injected 2 hours after either the *Candida* extract or the endotoxin of *E. coli* (Figure 13).

#### (f) Sensitization with Soluble Extract of *Candida albicans*

Twelve guinea pigs were injected intraperitoneally with various amounts of the soluble extract of *Candida albicans*. Eighteen days later varying amounts of the same material were injected intracardially to 6 of them, the remaining 6 guinea pigs were given the shocking dose of the extract intraperitoneal.

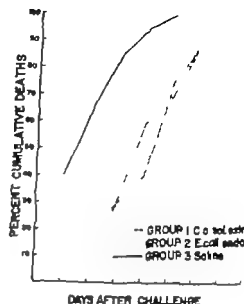
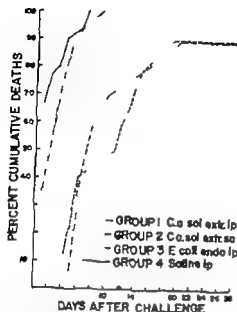


Fig. 10. Effect of 2 ip (Group 1: 20 mice) or sc (Group 2: 20 mice) injections of soluble extract of *Candida albicans* given on first (150 mg) and second (200 mg) day of experiment, and of 2 ip injections of *Escherichia coli* endotoxin (Group 3: 20 mice) given at the same times (25 µg each time) on prechallenge with 0.15 cc of a 1:100 dilution of a broth culture of *Salmonella enteritidis* given on the fourth day of the experiment. Controls (Group 4: 30 mice) were pretreated with injections of 0.5 cc of saline solution ip.

Fig. 11. Effect of an ip injection of 200 mg of soluble extract of *Candida albicans* (Group 1) and of 35 µg of *Escherichia coli* endotoxin (Group 2) on death rates of mice challenged with 0.2 cc of a 1:20 dilution of a broth culture of *Salmonella enteritidis* 5 days after pretreatment. Controls (Group 3) were pretreated with 0.5 cc of saline solution ip. Each group: 20 mice.

*Candida* extract and *E. coli* endotoxin was still present when the interval between pretreatment and challenge was 14 days (Figure 5) but no effect on the survival times could be detected when the interval was 30 days (Figure 6).

The survival time of mice was shortened when *E. coli* endotoxin was injected 3 hours (Figure 7) or 3 days (Figure 8) after the *Candida* injection. A similar result was obtained when the soluble extract of *C. albicans* was injected 3 hours after the infection with *C. albicans* (Figure 9).

#### (c) Changes in Nonspecific Resistance to *Salmonella enteritidis* Induced by Soluble Extract of *Candida albicans* and Endotoxin of *Escherichia coli*

Twenty mice were injected intraperitoneally with 150 mg of the soluble extract of *C.*

*Candida albicans* on the first day of the experiment. On the second day 200 mg of the same material was injected intraperitoneally. Another group of 20 animals was injected at the same time subcutaneously with the same amount of the *Candida* extract. A third group of 20 animals received 2 injections of 25 µg each of the endotoxin of *E. coli* intraperitoneally instead. A fourth group of 30 animals served as control and received physiologic saline solution intraperitoneally. Mice in all 4 groups were challenged with an intraperitoneal injection of 0.2 cc of a 1:20 dilution of 24-hour broth culture of *Salmonella enteritidis* on the fourth day of the experiment. Animals pretreated intraperitoneally with the *Candida* extract with *E. coli* endotoxin survived longer than the control which developed the infection and in third of them (20 of 30) died within 24 hours. The first mouse of the control group died 7 days

### (3) REVIEW OF LITERATURE

A detailed critical review of past experimental work may be desirable for better understanding of the successes and pitfalls of immunization against moniliasis, and of certain aspects of the pathogenicity of *Candida*. It is believed that most of these vaccination trials may be satisfactorily interpreted on the basis of the experimental findings reported in this paper which clarify some aspects of nonspecific host resistance, hyper sensitivity and localization of various immunologically active materials in distinct cell constituents of *Candida*. The limited past experimental findings in these fields have not been taken into account or correlated in planning immunization experiments in which the choice dosage timing and route of administration of both the antigens and the materials used for challenge are factors of utmost importance.

Comprehensive reviews of the subject of immunization in moniliasis are not available. Fischl (33) discussed earlier immunization trials while only three recent protection experiments were mentioned in the review of Levine (73). Reports on the effect of various microorganisms or their fractions on non-specific resistance to *Candida* are scattered in the literature. No systematic attempt has been made to explain the phenomena of enhancement or depression of resistance in such situations, an attempt which has been undertaken for

similar occurrences in bacterial infections (6, 8, 9, 62, 114-134).

A search of the earlier literature on moniliasis appeared generally more rewarding than a review of more recent articles on the subject. This applied not only to the experimental work but also to clinical and pathological descriptions. One of the best articles in the latter category was written by Vallée (142) as early as 1838, one year before the fungus of thrush was discovered. His work demonstrates that generalized monilism, a condition in which management based on immunological principles may be of value, had been a well known clinical entity more than a century before the discovery of antibiotics. The importance of disseminated moniliasis should not be underestimated today at a time in which enhancement of *Candida* infections by antibiotics and other modern drugs may play an important role in some parts of the world, while sanitary conditions probably not unlike those of Paris in 1838 may contribute to its development in other less developed regions.

Most of the important experimental findings concerning the immunology of *Candida* have been made more than quarter of a century ago. Concetti (14) based on a small experiment, postulated in 1900 that protection against moniliasis was possible by injections of cell walls of *Candida*. del Pont (16) has shown



Table 1 Attempts to sensitize guinea pigs with soluble extract of *Candida albicans* and with its cell walls

Guinea pig No.	Sensitizing Injection		Challenging Injection		Outcome
		mg.		mg.	
1	Sol. extr ip	5	Sol. extr ic <sup>1</sup>	50	2
2	Sol. extr ip	10	Sol. extr ic	100	Died
3	Sol. extr ip	10	Sol. extr ic	100	3
4	Sol. extr ip	50	Sol. extr ic	200	3
5	Sol. extr ip	100	Sol. extr ic	200	2
6	Sol. extr ip	100	Sol. extr ic	500	Died
7	Sol. extr ip	5	Sol. extr ip	50	2
8	Sol. extr ip	10	Sol. extr ip	100	3
9	Sol. extr ip	10	Sol. extr ip	100	2
10	Sol. extr ip	50	Sol. extr ip	200	No reaction
11	Sol. extr ip	100	Sol. extr ip	200	3
12	Sol. extr ip	100	Sol. extr ip	500	2
13	Cell walls ip	1	Cell walls ip	10	No reaction
14	Cell walls ip	5	Cell walls ip	25	No reaction
15	Cell walls ip	10	Cell walls ip	25	No reaction
16	Cell walls ip	10	Cell walls ip	25	No reaction
17	Cell walls p	10	Cell walls ip	50	No reaction
18	Cell walls ip	25	Cell walls ip	50	No reaction
19	None		Sol. extr ic	100	No reaction
20	None		Sol. extr ic	100	No reaction
21	None		Cell walls ip	25	No reaction
22	None		Cell walls ip	25	No reaction

The challenging injection was given 18 days after the sensitizing dose.

ic = intracardially 2 = moderate, 3 = severe anaphylactic shock.

ly All six guinea pigs given the intracardial injection developed symptoms of a moderate to severe anaphylactic shock within 1 to 3 minutes. The symptoms included ruffled hair, restlessness, cough, cyanosis, rubbing of nose, marked respiratory distress, convulsions, weakness. Two of the animals died within 5 minutes, the remaining 4 recovered.

Five of the six guinea pigs which received the challenging dose intraperitoneally developed symptoms of a moderate to severe anaphylactic shock but all of them recovered.

Six guinea pigs were injected intraperitoneally with various amounts of cell walls of

*Candida albicans*. On the eighteenth day of the experiment they received a challenging dose of the same material intraperitoneally. None of the animals developed signs of anaphylactic shock.

Two control animals, not previously sensitized, received 100 mg. of the soluble extract of *Candida albicans* intracardially. Two other non-sensitized guinea pigs were given an intraperitoneal injection of 25 mg. of cell walls of *C. albicans*. None of these four animals developed any symptoms of anaphylactic shock. These experiments are summarized in Table 1.

### (3) REVIEW OF LITERATURE

A detailed critical review of past experimental work may be desirable for better understanding of the successes and pit falls of immunization against monilliasis, and of certain aspects of the pathogenicity of *Candida*. It is believed that most of these vaccination trials may be satisfactorily interpreted on the basis of the experimental findings reported in this paper which clarify some aspects of nonspecific host resistance, hypersensitivity and localization of various immunologically active materials in distinct cell constituents of *Candida*. The limited past experimental findings in these fields have not been taken into account or correlated in planning immunization experiments in which the choice, dosage, timing and route of administration of both the antigens and the materials used for challenge are factors of utmost importance.

Comprehensive reviews of the subject of immunization in monilliasis are not available. Fischl (33) discussed earlier immunization trials while only three recent protection experiments were mentioned in the review of Levine (75). Reports on the effect of various microorganisms or their fractions on nonspecific resistance to *Candida* are scattered in the literature. No systematic attempt has been made to explain the phenomena of enhancement or depression of resistance in such situations, an attempt which has been undertaken for

similar occurrences in bacterial infections (6, 8, 11 62, 114 134).

A search of the earlier literature on monilliasis appeared generally more rewarding than a review of more recent articles on the subject. This applied not only to the experimental work but also to clinical and pathological descriptions. One of the best articles in the latter category was written by Valledix (142) as early as 1838, one year before the fungus of thrush was discovered. His work demonstrates that generalized monilliasis, a condition in which management based on immunological principles may be of value, had been a well known clinical entity more than a century before the discovery of antibiotics. The importance of disseminated monilliasis should not be underestimated today at a time in which enhancement of *Candida* infections by antibiotics and other modern drugs may play an important role in some parts of the world, while sanitary conditions probably not unlike those of Paris in 1838 may contribute to its development in other less developed regions.

Most of the important experimental findings concerning the immunology of *Candida* have been made more than a quarter of a century ago. Concetti (14) based on small experiment, postulated in 1930 that protection against monilliasis was possible by injections of cell walls of *Candida*. del Pont (16) has shown

in 1904 the feasibility of passive immunization. Kurotchkin and Lum (68) concluded in 1933 that sensitization was one of the possible mechanisms for generalization of *Candida* infections. Ninni and Fittipaldi (96) have noticed in 1934 increased susceptibility to *Candida* infection in animals given repeated frequent small doses of the fungus, a phenomenon apparently based on the resistance-influencing effect of the endotoxin like substance of *Candida*. No attempts have been made in the intervening period to correlate these findings or to utilize them for the management of moniliasis or any other fungus disease by immunological means.

The review of literature is divided into three sections, viz. nonspecific host resistance, allergy and its relation to immunity and specific immunity. Each of the reviewed experiments was classified under the heading which appeared to have played the most important role in inducing the described changes. Since several factors may have been responsible for the changes in some experiments this classification may only be an arbitrary one.

### (A) Nonspecific Host Resistance

The literature on the role of the fungi of the genus *Candida* in nonspecific host resistance is reviewed here since its knowledge is helpful in evaluating the experimental work on specific immunity which will be discussed later in this paper. Much influence on the ultimate outcome of immunization appears to be exerted by the endotoxin like substance of the soluble fraction which, depending

on timing either enhances or depresses the resistance to *Candida* and may thus enhance or nullify the effect of the protective antigen which, as was shown by own experiments, apparently resides in the cell walls of *Candida*.

*Candida* has been used either as an organism to which nonspecific resistance was induced, or an agent or source of substances for the enhancement or depression of resistance to other microorganisms.

Studies in which *Candida* was used in an animal both as a modifying agent and an infecting organism, and in which there is a relatively short interval between pretreatment and challenge, are included here rather than in the section on specific immunization. It is probable that the change in the course of the infection in such experiments is not based on mechanisms of specific immunity.

The experiments dealing with nonspecific resistance may be divided into two groups.

- 1) The first group includes experiments in which the agent used for modification of the infection was either a dead microorganism, an extract therefrom or a substance unrelated to a microorganism. In these experiments the agent may be accurately measured and certain variables inherent in the use of a living and multiplying agent are eliminated. This group may be subdivided into the following sections:

- (a) Experiments in which enhancement or depression of nonspecific resistance against a *Candida* infection was induced using various dead microorganisms or their products or substances not derived from microorganisms.

(b) Studies in which dead *Candida* or its products were used to enhance or depress resistance against a *Candida* infection.

(c) Experiments in which dead fungi of the genus *Candida* or their products were used to induce enhancement or depression of resistance against other microorganisms in animals.

(ii) In the second group are included experiments in which living microorganisms were administered in two or more injections. The organism injected in one or all of them was *Candida*. Injections of two different organisms were given either simultaneously or at varying intervals between each other.

Because of varying rates of multiplication, production and liberation of substances effective in modifying infections different microorganisms at different dosage levels and routes of administration may cause response which, unlike in the experiments of the first group, cannot be easily predicted. In some instances the substance from the first injection, if more potent, may nullify the effect of that from the second injection, or vice versa. The sum of the inoculum determining among other factors the rate of spread of the microorganism in the animal, may conceivably influence the time of maximum liberation of the endotoxin-like substance deciding thus whether the infection will be enhanced or depressed. This second group may be subdivided into the following sections:

(a) Studies of the effects caused by injections of living *Candida* and other living microorganisms in animals. Changes in the course of such mixed

infections may be interpreted as enhancement or depression of nonspecific host resistance induced by endotoxin-like substances from either *Candida* or the bacterial organism in question, or the effect of antibacterial or antifungal substances which these organisms may produce.

(b) Studies of the effects of living *Candida* cells on the resistance to a *Candida* infection.

(i) (a) *Effect of Dead Organisms their Products or Substances not Derived from Micro-organisms on Nonspecific Resistance to Candida*

Products of bacterial organisms and fungi, as well as sterile milk, were used to modify resistance to *Candida* infections.

Gale and Sandoval (37) injected various concentrations of heat-killed cells of *Escherichia coli* or 1.0 to 100  $\mu$ g of *E. coli* endotoxin 30 minutes after an intra-cerebral injection of *Candida albicans* into mice and found that their survival time was shortened as compared to control mice injected with *Candida* alone.

These experiments demonstrate that, similarly as in bacterial infections, endotoxins stimulate the growth of fungi when injected shortly after a fungal challenge.

Hastenecker and Mitchell (41) injected mice intraperitoneally with 30  $\mu$ g of the endotoxin of *Salmonella enteritidis* 1 to 14 days before an intravenous challenge with  $10^7$  to  $2 \times 10^7$  cells of *Candida albicans*. The survival times of controls were less than 24 hours. The

lives of mice pretreated either 1 or 6 days before challenge were prolonged while no essential prolongation of survival times was noted in mice treated 3, 10 or 14 days before challenge. Mice given 20  $\mu$ g of the endotoxin of *Salmonella typhosa* or *S. enteritidis* 6 days before and 30  $\mu$ g one day before challenge lived somewhat longer than those receiving a single injection of endotoxin. Animals given 20  $\mu$ g of *S. enteritidis* endotoxin 7 days, and 30  $\mu$ g 6 days and 1 day before challenge survived the longest. Mice treated with  $10^7$  viable cells of *C. albicans* and 30  $\mu$ g of *S. enteritidis* endotoxin intraperitoneally 5 days before challenge lived somewhat longer than those given the endotoxin alone. The same was true of mice receiving  $10^7$  viable cells of *C. albicans* 6 days and 30  $\mu$ g of *S. enteritidis* endotoxin 1 day before challenge. Prolongation of survival times after pretreatment with endotoxin was observed in mice 6 or 12 weeks old but 9-week-old mice failed to respond.

Partial protection of mice pretreated with endotoxin 1 or 6 days before challenge is in agreement with own experiments in which this effect was observed when endotoxin of *Escherichia coli* or the soluble extract of *C. albicans* was injected 5, 8 or 14 days before challenge with *C. albicans* (Figures 2, 3, 4 and 5). Hasenclever and Mitchell did not see however any effect when the interval between pretreatment and challenge was 3, 10 or 14 days.

Other experiments of the same authors show that there was no essential change in the survival times of mice given 1 mg of nonviable *Mycobacterium butyricum* intraperitoneally or those receiving 0.1

ml. of oil by the same route 6 days before challenge. The intraperitoneal administration of the complete Freund's adjuvant containing 1 mg of *M. butyricum* in 0.1 ml of oil prolonged the survival times of the mice somewhat.

Scherr (129) injected mice intraperitoneally with  $10^8$  formalin treated cells of *Saccharomyces cerevisiae* every other day except for a 5-day rest period after the sixth day. The injections were started 24 hours after an intraperitoneal administration of  $5 \times 10^6$  cells of *Candida albicans*. When the mice were sacrificed 20 days after the infection with *Candida* it was found that the degree of dissemination of monilia was much more pronounced in animals injected with formalin-treated *Saccharomyces* than in untreated controls which received only *Candida*.

In another experiment mice received ten daily intraperitoneal injections of a supernatant remaining after grinding of an aqueous suspension of *Saccharomyces* with sand in a mortar. The supernatant was not concentrated. This treatment was started 24 hours after an intraperitoneal injection of one half the amount of the *Candida* suspension used in the preceding experiment. Treatment with this material had no effect on the dissemination of monilia.

Another group of mice received  $5 \times 10^6$  cells of *C. albicans* intraperitoneally. Twenty-four hours later injections of concentrated culture filtrates of *S. cerevisiae* were started and repeated every other day for a total of 10 injections. When the mice were sacrificed 20 days after the injection of *Candida* it was found that the degree of dis-

termination of moniliasis was about the same as in untreated controls which received only *Candida*.

The experiments with dead cells of *S. cerevisiae* given repeatedly to mice after they had been infected with *Candida* demonstrate the same dependence of the effect on timing which was seen in the work of Gale and Sandoval (36) and in our experiments (Figures 7 and 8). The finding that small amounts of an extract prepared by grinding of *S. cerevisiae* which was not concentrated did not have any effect on the resistance to *Candida* may have been caused by the fact that too little of the active principle was injected. It is not surprising that culture filtrates of *S. cerevisiae* were ineffective. If an analogy exists between the location of the resistance-influencing substance of *Candida* and *S. cerevisiae* the active principle would be expected to be present in a soluble cell fraction of *S. cerevisiae*.

Whitney Parfentjev and Whitney (149) injected mice intraperitoneally with single dose of malucidin, a yeast extract, 24 or 48 hours before an intraperitoneal injection of 100,000,000 cells of *Candida albicans*. Five hours before the challenge dose of *Candida* the mice received 2 mg of aureomycin intraperitoneally. Without this antibiotic pretreatment the fungal challenge was not lethal. Pretreatment with malucidin of aureomycin-enhanced infection protected many mice during the acute phase of moniliasis. Forty-two out of 100 mice given various batches and dosage levels of malucidin were still living after 12 days while all control mice which were given aureomycin and *Candida* died.

This protection against *Candida* may also be due to the same mechanism. It is apparently not based on any antibiotic effect since no inhibition of growth was noted when *C. albicans* was incubated with malucidin *in vitro*. This and the preceding experiment of Scherr (129) point to the possibility that *Saccharomyces cerevisiae* contains a substance similar to that present in the soluble fraction of *Candida* or in gram-negative organisms, which is effective in influencing nonspecific resistance. It is perhaps interesting that such substances may also be present in nonpathogenic fungal organisms.

Hasenclever and Mitchell (40-41) injected mice intraperitoneally with 1 mg of formalin-killed mechanically disintegrated spherules of *Coccidioides immitis* six days before they challenged them intravenously with  $10^7$  cells of *Candida albicans*. The life of pretreated mice was somewhat prolonged as compared with mice which received *Candida* challenge only.

This experiment points to the possibility that *Coccidioides immitis* may contain an endotoxin-like substance, similar to that of *Candida*, which is capable of influencing nonspecific resistance.

Fischer and Horbach (31) pretreated 29 mice with 0.5 cc. of sterile milk intraperitoneally 24 hours before they challenged them by the same route with 0.2 cc. of a 4% suspension of *Candida albicans*. Only six of the pretreated mice died in 3 weeks while there were 15 deaths among 30 controls which received the challenge dose only.

In this experiment milk appeared to have protective effect. Such a property

lives of mice pretreated either 1 or 6 days before challenge were prolonged while no essential prolongation of survival times was noted in mice treated 3, 10 or 14 days before challenge. Mice given 20  $\mu$ g of the endotoxin of *Salmonella typhosa* or *S. enteritidis* 8 days before and 30  $\mu$ g one day before challenge lived somewhat longer than those receiving a single injection of endotoxin. Animals given 20  $\mu$ g of *S. enteritidis* endotoxin 7 days, and 30  $\mu$ g 6 days and 1 day before challenge survived the long test. Mice treated with  $10^7$  viable cells of *C. albicans* and 30  $\mu$ g of *S. enteritidis* endotoxin intraperitoneally 6 days before challenge lived somewhat longer than those given the endotoxin alone. The same was true of mice receiving  $10^7$  viable cells of *C. albicans* 6 days and 30  $\mu$ g of *S. enteritidis* endotoxin 1 day before challenge. Prolongation of survival times after pretreatment with endotoxin was observed in mice 6 or 12 weeks old, but 9-week-old mice failed to respond.

Partial protection of mice pretreated with endotoxin 1 or 6 days before challenge is in agreement with own experiments in which this effect was observed when endotoxin of *Escherichia coli* or the soluble extract of *C. albicans* was injected 5, 8 or 14 days before challenge with *C. albicans* (Figures 2, 3, 4 and 5). Hasenclever and Mitchell did not see, however, any effect when the interval between pretreatment and challenge was 3, 10 or 14 days.

Other experiments of the same authors show that there was no essential change in the survival times of mice given 1 mg. of nonviable *Mycobacterium butyricum* intraperitoneally or those receiving 0.1

ml. of oil by the same route 6 days before challenge. The intraperitoneal administration of the complete Freund's adjuvant containing 1 mg. of *M. butyricum* in 0.1 ml. of oil prolonged the survival times of the mice somewhat.

Scherr (129) injected mice intraperitoneally with 10 formalin-treated cells of *Saccharomyces cerevisiae* every other day except for a 5-day rest period after the sixth day. The injections were started 24 hours after an intraperitoneal administration of  $5 \times 10^5$  cells of *Candida albicans*. When the mice were sacrificed 20 days after the infection with *Candida* it was found that the degree of dissemination of moniliasis was much more pronounced in animals injected with formalin-treated *Saccharomyces* than in untreated controls which received only *Candida*.

In another experiment mice received ten daily intraperitoneal injections of a supernatant remaining after grinding of an aqueous suspension of *Saccharomyces* with sand in a mortar. The supernatant was not concentrated. This treatment was started 24 hours after an intraperitoneal injection of one half the amount of the *Candida* suspension used in the preceding experiment. Treatment with this material had no effect on the dissemination of moniliasis.

Another group of mice received  $5 \times 10^5$  cells of *C. albicans* intraperitoneally. Twenty-four hours later injections of concentrated culture filtrates of *S. cerevisiae* were started and repeated every other day for a total of 10 injections. When the mice were sacrificed 20 days after the injection of *Candida* it was found that the degree of dis-

The death rate of pretreated mice was somewhat lower than that of the controls. If any significance may be attached to this experiment, the difference may be explained by the presence of the endotoxin-like substance in the first injection. However possibly because of the short time interval between the first and second injection, the protection was not too pronounced.

Hasenclever and Mitchell (40) injected mice intraperitoneally or intravenously with  $10^7$  or  $5 \times 10^7$  heat killed cells of *Candida albicans* and challenged them 6 days later intravenously with  $10^7$  viable cells of the same fungus. There was not much difference in the survival time between pretreated mice and controls except for a group pretreated intraperitoneally with the higher dose,  $5 \times 10^7$  dead cells, in which the survival time was somewhat prolonged.

This experiment may also be interpreted on the basis of the protective effect of the endotoxin-like substance contained in the dead cells of *C. albicans* when administered several days before challenge. The number of cells in the pretreatment dose may have been somewhat low which may explain some effect only with  $5 \times 10^7$  heat killed cells. Although great individual variations in the response of mice may be expected from one experiment to another it should be noted that Scherr (129) used about eight doses of  $10^6$  dead cells each, and Fischer and Horbach (31) injected  $10^6$  dead cells once. In own experiments 200 mg. of soluble extract which was found effective for the enhancement or depression of nonspecific resistance was prepared from about 20,000,000,000 cells.

Isenberg, Allerhand and Berkman (34) injected mice intravenously with 0.2 cc. of an aqueous fraction isolated by ethanol-ethyl ether or phenol extraction of intact cells of *Candida albicans*. Six days later they challenged them intravenously with 10 million cells of the homologous strain. The pretreated mice were protected while the controls died. The number of experimental animals or the length of the observation period were not given.

The experiments in this group demonstrate the resistance-influencing effects of formalin-treated or heat-killed cells of *Candida* or a *Candida* extract on *Candida* infections. Repeated injections of dead cells given after infection with *Candida albicans* enhance the growth of the fungus. Dead cells of *C. albicans* or its extract given several days before challenge have a protective effect. These findings are confirmed by own experiments in which a soluble extract of *C. albicans* was used (Figures 2, 3, 4, 5 and 9). The resistance-influencing activities of the endotoxin-like substance of *Candida* are apparently not destroyed after exposure to heat, as evidenced by the experiments of Fischer and Horbach (31) and Hasenclever and Mitchell (40) who used heat killed cells of *Candida* and by own experiments in which heat-treated extract of *Candida* was effective (Figure 4).

(i) Effect of Dead *Candida* or its Product on Resistance to other Microorganisms

Ruicillo et al. (121-122) have prepared material from *Candida albicans* by treating yeast cells with trichloroacetic



of milk has been reported in relation to bacterial infections (8)

The experiments in this group demonstrate enhancement of *Candida* infections by the injection of heat killed cells of a gram negative organism, *Escherichia coli* or its endotoxin given shortly after the infection. Own experiments with *E. coli* endotoxin confirm these findings (Figure 7) and furnish supplemental data concerning the dependence of the effect on the time of administration of the endotoxin (Figures 2 5 8 and 8). Partial protection of mice pretreated 1 or 6 days before challenge with endotoxin of *Salmonella enteritidis* or *S. typhosa* was also demonstrated and is in agreement with own findings (Figures 2 5 and 6). No protection was noted however when the interval was 9 10 or 14 days. Repeated injections of the endotoxins given 7 6 and 1 day before challenge offered a somewhat better protection.

Nonviable *Mycobacterium butyricum* given intraperitoneally 5 days before challenge with *Candida* did not prolong the survival time while the complete Freund's adjuvant containing this organism in oil offered some protection.

Other experiments in this group demonstrated enhancement of *Candida* infections by injections of dead cells of a nonpathogenic fungus *Saccharomyces cerevisiae* given shortly after an infection with *Candida* and a partial protection against a *Candida* infection by injections of an extract from this yeast malucidin given 24 hours or 48 hours before challenge. Disintegrated spherules of a pathogenic fungus, *Coccidioides immitis* given several days before chal-

lenge with *Candida* protected mice somewhat against this challenge. The dependence of the effect on the time of administration of the mentioned fungal materials was in agreement with own experiments in which *E. coli* endotoxin and *Candida* extract were used (Figures 2, 3 4 7 8 and 9). Sterile milk given 24 hours before challenge with *Candida* also caused a prolongation of life in mice

#### (1) (b) Effect of Dead *Candida* or its Products on Nonspecific Resistance to *Candida* Infections

Scherr (129) injected mice repeatedly with  $10^8$  formalin treated cells of *Candida albicans* intraperitoneally applying the same schedule as in a previously described experiment with formalin-treated cells of *Saccharomyces cerevisiae* (page 18). The injections were started 24 hours after an intraperitoneal injection of  $5 \times 10^5$  cells of *C. albicans*. The resulting degree of dissemination of the *Candida* infection was much more pronounced in mice injected with formalin-treated *C. albicans* than in untreated controls which received the infecting dose of *Candida* only.

Fischer and Horbach (31) pretreated 30 mice with 0.2 cc of a 4% suspension of heat killed cells intraperitoneally 24 hours before they challenged them by the same route with a suspension of living *Candida* containing the same number of cells as the first injection. Ten of them died in 3 weeks while there were 15 deaths among 30 controls which received the challenge dose only and deaths among 15 mice receiving pre-treatment only and no challenge.

of this material. This impression seems supported by the finding of Yamabayashi that a *Candida* extract enhanced the bacterial challenge only when injected within about 12 hours preceding it, signifying possibly that the resistance-influencing substance was more promptly available from the extract than from intact dead cells. Unlike Ruscidlo et al. (120, 11-122) Yamabayashi (15) found this mechanism to be operative only in gram-negative but not in gram-positive bacterial organisms. In the experience of Yamabayashi the growth of *Staphylococcus* or *Streptococcus* was not enhanced.

Mankiewicz and Litvak (78) have inoculated by subcutaneous route 20 guinea pigs simultaneously with *Mycobacterium tuberculosis* and 1 ml. of a 5% solution of a heat-stable polysaccharide fraction of *Candida albicans*. This was prepared by chemical extraction from supernatant after mechanical disintegration of the yeast cells of the fungus. Twenty controls were injected with *M. tuberculosis* alone.

Six of 20 guinea pigs injected with *M. tuberculosis* and the *Candida* polysaccharide died within 6 weeks while 2 of 20 animals injected with *M. tuberculosis* alone died of an unrelated bacterial infection. The rate of tissue invasion with *M. tuberculosis* in animals injected with the *Candida* polysaccharide and *M. tuberculosis* was higher than in those inoculated with the tubercle bacilli alone.

Mankiewicz and Litvak (78) reported that the chemical nature of the polysaccharide from *C. albicans* was similar

to bacterial dextrans which were shown to stimulate the growth of enterobacteria in vivo.

Mankiewicz, Stackiewicz and Litvak (79) also found that the polysaccharide fraction of *C. albicans* promoted the growth of *M. tuberculosis* in vitro.

It is possible that the shortened survival time of guinea pigs injected with *M. tuberculosis* and the polysaccharide of *C. albicans* may have been due to the presence of an endotoxin-like substance of *Candida* which, as in previously described experiments with other bacterial organisms, may have decreased the resistance of the animals to *M. tuberculosis*. It is also possible that the growth of the tubercle bacillus may have been promoted by another mechanism as well, which was independent of lowering of nonspecific resistance by an endotoxin-like substance in vivo and may have been based on the in vitro growth-promoting effect of the polysaccharide fraction of *C. albicans* described by Mankiewicz, Stackiewicz and Litvak (79).

The experiments in this group demonstrate that an extract of *Candida albicans* injected simultaneously with *Salmonella typhosa* or *Staphylococcus aureus* or an extract of *Candida krusei* injected simultaneously with *Staphylococcus aureus* in the experiments of Ruscidlo et al. (120, 121-122) enhances the bacterial infection. Heat-killed cells of *C. albicans* in the experiments of Yamabayashi (152) injected less than 48 hours before challenge with *Proteus* or *Pseudomonas* or an extract of the fungus prepared by trypsin digestion and

acid, neutralizing them with sodium hydroxide and precipitating with acetone. Extraction of insoluble polysaccharides was done with  $\text{Na}_2\text{HPO}_4$ . After filtering and discarding of the solution the remaining yeast was suspended in water and alkalized with sodium hydroxide. Digestion of the remainder was done with trypsin and the sediment was boiled in water. Boiling in water was repeated after suspension of the sediment in alcohol. The dry residue was ground in a mortar. This material which the authors called zymosan presumably originated in cell walls of *Candida*.

Mice were injected with mixtures of 5 mg of this material and varying numbers of 1 million to 2,000 million cells of *Salmonella typhosa* intraperitoneally. In a control group only 2,000 million bacteria were lethal to mice. In the groups injected with the mixtures of *Salmonella* and the extract from *Candida* even mice receiving the smallest doses of bacteria died.

In another series of experiments mice were injected with a mixture of *Staphylococcus aureus* and 5 mg of the described *Candida* extract. All these mice died within the first 24 hours while in a control group of mice receiving *Candida* extract only all animals survived.

Rzucidlo Stachów Nowakowska and Kubiś (120) injected 5 mg of a similarly prepared extract from *Candida Krusei* together with a suspension of *Staphylococcus aureus* intraperitoneally into mice. All 10 mice injected with this mixture died within 18 hours while 10 controls receiving *Staphylococcus* alone survived for more than 48 hours.

Mice receiving the cell extract of *C. Krusei* alone remained alive during the 10 day observation period.

Yamabayashi (157) injected a suspension of  $50 \times 10^7$  heat killed cells of *Candida albicans* which was followed 2 to 48 hours later by the administration of 1/10 of the minimal lethal dose of either *Proteus* or *Pseudomonas*. The death rates were highest when bacterial organisms were injected 2 hours after *Candida* and almost no effect on the death of mice was seen when bacterial organisms were injected 48 hours after heat killed *Candida*.

Yamabayashi also prepared a zymosan-like substance from *C. albicans* by trypsin digestion. It was a light-gray powder insoluble in water mainly composed of carbohydrate. Mice were injected with 2 mg of this substance intraperitoneally and the injection was followed 1/2 to 48 hours later by an intraperitoneal administration of *Proteus vulgaris*, *Pseudomonas aeruginosa* or *Escherichia coli*. The death rates were very high when mice were infected up to 12 hours after the injection of the *Candida* extract. This effect was less pronounced with *E. coli* than it was with the first two organisms. No effects were seen after injections of *Staphylococcus aureus* or *Streptococcus haemolyticus*.

The finding that dead *Candida* cells were effective in enhancing the bacterial infection when given up to about 48 hours before bacterial challenge may be due to the possibility that the endotoxin like substance may be released rather slowly from dead cells, and that peak blood levels of endotoxin may be reached many hours after the injection.

of this material. This impression seems supported by the finding of Yamabayashi that a *Candida* extract enhanced the bacterial challenge only when injected within about 12 hours preceding it, signifying possibly that the resistance-influencing substance was more promptly available from the extract than from intact dead cells. Unlike Ruscdlo et al. (120, 121, 122) Yamabayashi (152) found this mechanism to be operative only in gram-negative but not in gram-positive bacterial organisms. In the experience of Yamabayashi the growth of *St. ph. aureus* or *Streptococcus* was not enhanced.

Maniewicz and Litvak (78) have inoculated by subcutaneous route 20 guinea pigs simultaneously with *Mycobacterium tuberculosis* and 1 ml. of a 5% solution of heat-stable polysaccharide fraction of *Candida albicans*. This was prepared by chemical extraction from a supernatant after mechanical disintegration of the yeast cells of the fungus. Twenty controls were injected with *M. tuberculosis* alone.

Six of 20 guinea pigs injected with *M. tuberculosis* and the *Candida* polysaccharide died within 6 weeks while 2 of 20 animals injected with *M. tuberculosis* alone died of an unrelated bacterial infection. The rate of tissue invasion with *M. tuberculosis* in animals injected with the *Candida* polysaccharide and *M. tuberculosis* was higher than in those inoculated with the tubercle bacilli alone.

Maniewicz and Litvak (78) reported that the chemical nature of the polysaccharide from *C. albicans* was similar

to bacterial dextrans which were shown to stimulate the growth of enterobacteria *in vivo*.

Maniewicz, Stackiewicz and Litvak (79) also found that the polysaccharide fraction of *C. albicans* promoted the growth of *M. tuberculosis* *in vitro*.

It is possible that the shortened survival time of guinea pigs injected with *M. tuberculosis* and the polysaccharide of *C. albicans* may have been due to the presence of an endotoxin-like substance of *Candida* which, as in previously described experiments with other bacterial organisms, may have decreased the resistance of the animals to *M. tuberculosis*. It is also possible that the growth of the tubercle bacillus may have been promoted by another mechanism as well, which was independent of lowering of nonspecific resistance by an endotoxin-like substance *in vivo* and may have been based on the *in vitro* growth-promoting effect of the polysaccharide fraction of *C. albicans* described by Maniewicz, Stackiewicz and Litvak (79).

The experiments in this group demonstrate that an extract of *Candida albicans* injected simultaneously with *Salmonella typhosa* or *Staphylococcus aureus* or an extract of *Candida lusitana* injected simultaneously with *Staphylococcus aureus* in the experiments of Ruscdlo et al. (120, 121, 122) enhances the bacterial infection. Heat-killed cells of *C. albicans* in the experiments of Yamabayashi (152) injected less than 48 hours before challenge with *P. otitis* or *Pseudomonas*, or an extract of the fungus prepared by trypsin digestion and

acid, neutralizing them with sodium hydroxide and precipitating with acetone. Extraction of insoluble polysaccharides was done with  $\text{NaHPO}_4$ . After filtering and discarding of the solution the remaining yeast was suspended in water and alkalized with sodium hydroxide. Digestion of the remainder was done with trypsin and the sediment was boiled in water. Boiling in water was repeated after suspension of the sediment in alcohol. The dry residue was ground in a mortar. This material which the authors called zymosan presumably originated in cell walls of *Candida*.

Mice were injected with mixtures of 5 mg of this material and varying numbers of 1 million to 2 000 million cells of *Salmonella typhosa* intraperitoneally. In a control group only 2 000 million bacteria were lethal to mice. In the groups injected with the mixtures of *Salmonella* and the extract from *Candida* even mice receiving the smallest doses of bacteria died.

In another series of experiments mice were injected with a mixture of *Staphylococcus aureus* and 5 mg of the described *Candida* extract. All these mice died within the first 24 hours while in a control group of mice receiving *Candida* extract only all animals survived.

Kruciklo Stachów, Nowakowaka and Kubica (120) injected 5 mg of a similarly prepared extract from *Candida Krusei* together with a suspension of *Staphylococcus aureus* intraperitoneally into mice. All 10 mice injected with this mixture died within 18 hours while 10 controls receiving *Staphylococcus* alone survived for more than 48 hours.

Mice receiving the cell extract of *C. Krusei* alone remained alive during the 10 day observation period.

Yamabayashi (152) injected a suspension of  $50 \times 10^7$  heat killed cells of *Candida albicans* which was followed 2 to 48 hours later by the administration of 1/10 of the minimal lethal dose of either *Proteus* or *Pseudomonas*. The death rates were highest when bacterial organisms were injected 2 hours after *Candida* and almost no effect on the death of mice was seen when bacterial organisms were injected 48 hours after heat killed *Candida*.

Yamabayashi also prepared a zymosan-like substance from *C. albicans* by trypsin digestion. It was a light-gray powder insoluble in water mainly composed of carbohydrate. Mice were injected with 2 mg of this substance intraperitoneally and the injection was followed 1/2 to 48 hours later by an intraperitoneal administration of *Proteus vulgaris*, *Pseudomonas aeruginosa* or *Escherichia coli*. The death rates were very high when mice were infected up to 12 hours after the injection of the *Candida* extract. This effect was less pronounced with *E. coli* than it was with the first two organisms. No effects were seen after injections of *Staphylococcus aureus* or *Streptococcus haemolyticus*.

The finding that dead *Candida* cells were effective in enhancing the bacterial infection when given up to about 48 hours before bacterial challenge may be due to the possibility that the endotoxin-like substance may be released rather slowly from dead cells, and that peak blood levels of endotoxin may be reached many hours after the injection.

substances which may have mutually stimulated the growth of the injected microorganisms by the mechanism of nonspecific immunity. These findings are in agreement with the results of Yamabayashi (152) who confirmed and amplified them by including some additional organisms in his tests. Yamabayashi injected simultaneously groups of 20-50 mice by intraperitoneal route with varying amounts of *Candida albicans* and 1/10 of the minimal lethal dose of either *Proteus vulgaris*, *Pseudomonas aeruginosa* or *Escherichia coli*. All death rates were computed 7 days after the injections. The author found that the death rates of mice with simultaneous infections were considerably higher than those in which *Candida* alone was injected. This effect was more pronounced with *Proteus* and *Pseudomonas* than with *E. coli*. When gram-positive organisms, viz. *Staphylococcus aureus*, *Streptococcus hemolyticus* or *Bacillus subtilis* were injected simultaneously with *Candida* no increase of death rates was found.

In another series of experiments *Proteus* or *Pseudomonas* were injected at intervals between 2 and 48 hours after an injection of *Candida*. The death rates were highest when bacterial organisms were injected 2 hours after *Candida* and almost no effect on the death of mice was seen when bacterial organisms were injected 48 hours after *Candida*.

In other experiments it has been shown that there was no difference in colony counts between the mixed cultures and cultures of individual organisms.

The finding of Yamabayashi (152) that the mixed infection of *Candida* and

*Escherichia coli* caused fewer deaths than that of *Candida* and *Proteus* or *Pseudomonas* is further elucidated by the experiments of Gale and Sandoval (37). These authors injected into mice sublethal amounts of living *Escherichia coli* before or after an injection of a lethal concentration of *Candida*. The injections of *Candida* were always given intravenously, those of *E. coli* either intraperitoneally or intravenously.

Intravenous inoculation of  $1.8 \times 10^6$  of *Candida* alone killed all mice within 24 hours. When  $3.7 \times 10^7$  cells of *E. coli* were injected intravenously 30 minutes before the lethal *Candida* injection, 3 of 7 mice survived for 6 days, at which time they were sacrificed. When *E. coli* was given intraperitoneally under otherwise identical conditions 2 of 7 mice survived for 6 days. Intravenous inoculation of *E. coli* after the *Candida* injection was ineffectual in delaying the time of death while some protective activity was produced by the intraperitoneal inoculation of *E. coli* 30 or 150 minutes after *Candida*. This protective activity was produced by *E. coli* only within a narrow range of concentration ( $10^7$  and  $10^8$ ). For all concentrations of *E. coli* the survival time after administration of dead bacilli was shorter than after administration of the living organisms (page 17).

Gale and Sandoval (37) found also that *E. coli* was very effective in inhibiting the *in vitro* growth of *Candida albicans*. The greater the concentration of *E. coli* the more effective was the inhibition of *Candida*.

It appears probable that an antibiotic substance produced by *E. coli* may be

injected less than 12 hours before challenge with *Proteus Pseudomonas* or *E. coli* enhances the bacterial infection with the former 2 organisms and to a lesser extent with *E. coli*. Yamabayashi found no effect on the infection with *Staphylococcus* or *Streptococcus*. A simultaneous injection of a soluble polysaccharide of *C. albicans* enhanced the tuberculous infection in the experiments of Mankiewicz and Litvak (78).

It is possible that at least a part of the enhancing effect in these experiments, with the limitations mentioned earlier was due to the endotoxin-like substance of *C. albicans* or a similar substance of *C. Krusei*. A similar effect was illustrated by own experiments in which the survival time of mice injected with the soluble extract of *C. albicans* 2 hours before challenge with *Salmonella enteritidis* was shorter than in the controls (Figure 12).

As long however as the chemical nature of the own endotoxin-like substance of *Candida* and of the various above mentioned extracts is unknown it is impossible to determine whether the chemically extracted preparations of Ruzicido et al. (120, 121, 122) and Yamabayashi (152) actually contained the same resistance influencing substance which is present in the own soluble extract from *Candida*. The preparation of Mankiewicz and Litvak (78) was, similarly as the own soluble extract, prepared from the supernatant remaining after disintegration of yeast cells, so that most of the cell wall material was apparently excluded from both preparations.

### (ii) (a) Effect of Double Infections with *Candida* and Another Organism on Nonspecific Resistance

When evaluating the following group of experiments it has to be realized that since the two organisms are living both may be capable of producing constantly an endotoxin-like substance. They may however also produce antibiotic substances which may influence the development of the mixed infection.

Donomae and Kawamori (18) infected mice simultaneously with  $50 / 10^6$  cells of *Candida albicans* and 1/10 of the minimal lethal dose of *Proteus vulgaris*. About 50 % of controls injected with *Candida* only were dead at the end of 7 days while all mice with the mixed infection succumbed in 3 days. Another group of mice was infected simultaneously with  $100 / 10^6$  cells of *C. albicans* and 1/20 of the minimal lethal dose of *Proteus vulgaris*. About 20 % of controls injected with *Candida* only were dead after 7 days while all mice with the mixed infection died in 3 days. Similar results were obtained in a mixed infection with *C. albicans* and *Pseudomonas aeruginosa*. Death rates of mice injected simultaneously with *C. albicans* and 1/10 of the minimal lethal dose of either *Staphylococcus*, *Streptococcus hemolyticus*, *Escherichia coli* or *Bacillus subtilis* did not differ from those of mice which received *C. albicans* only.

The enhancement in a simultaneous infection with *Candida* and *P. vulgaris*, *Pseudomonas* was apparently due to liberation, by both *Candida* and the bacterial organism, of endotoxin-like

substances which may have mutually stimulated the growth of the injected microorganisms by the mechanism of nonspecific immunity. These findings are in agreement with the results of Yamabayashi (152) who confirmed and amplified them by including some additional organisms in his tests. Yamabayashi injected simultaneously groups of 20-30 mice by intraperitoneal route with varying amounts of *Candida albicans* and 1/10 of the minimal lethal dose of either *Proteus vulgaris*, *Pseudomonas aeruginosa* or *Escherichia coli*. All death rates were computed 7 days after the injections. The author found that the death rates of mice with simultaneous infections were considerably higher than those in which *Candida* alone was injected. This effect was more pronounced with *Proteus* and *Pseudomonas* than with *E. coli*. When gram-positive organisms, viz. *Staphylococcus aureus*, *Streptococcus hemolyticus* or *Bacillus subtilis* were injected simultaneously with *Candida* no increase of death rates was found.

In another series of experiments *Proteus* or *Pseudomonas* were injected at intervals between 2 and 48 hours after an injection of *Candida*. The death rates were highest when bacterial organisms were injected 2 hours after *Candida* and almost no effect on the death of mice was seen when bacterial organisms were injected 48 hours after *Candida*.

In two experiments have shown that there was no difference in colony counts between the mixed cultures and cultures of individual organisms.

The findings of Yamabayashi (152) that the mixed infection of *Candida* and

*Escherichia coli* caused fewer deaths than that of *Candida* and *Proteus* or *Pseudomonas* is further elucidated by the experiments of Gale and Sandoval (37). These authors injected into mice sublethal amounts of living *Escherichia coli* before or after an injection of a lethal concentration of *Candida*. The injections of *Candida* were always given intravenously those of *E. coli* either intraperitoneally or intravenously.

Intravenous inoculation of  $1.8 \times 10^8$  of *Candida* alone killed all mice within 24 hours. When  $3.7 \times 10^8$  cells of *E. coli* were injected intravenously 30 minutes before the lethal *Candida* injection, 3 of 7 mice survived for 6 days, at which time they were sacrificed. When *E. coli* was given intraperitoneally under otherwise identical conditions 2 of 7 mice survived for 6 days. Intravenous inoculation of *E. coli* after the *Candida* injection was ineffective in delaying the time of death while some protective activity was produced by the intraperitoneal inoculation of *E. coli* 30 or 150 minutes after *Candida*. This protective activity was produced by *E. coli* only within a narrow range of concentration ( $10^8$  and  $10^9$ ). For all concentrations of *E. coli* the survival time after administration of dead bacilli was shorter than after administration of the living organisms (page 17).

Gale and Sandoval (37) found also that *E. coli* was very effective in inhibiting the *in vitro* growth of *Candida albicans*. The greater the concentration of *E. coli* the more effective was the inhibition of *Candida*.

It appears probable that an antibiotic substance produced by *E. coli* may be



injected less than 12 hours before challenge with *Proteus Pseudomonas* or *E. coli* enhances the bacterial infection with the former 2 organisms, and to a lesser extent with *E. coli*. Yamabayashi found no effect on the infection with *Staphylococcus* or *Streptococcus*. A simultaneous injection of a soluble polysaccharide of *C. albicans* enhanced the tuberculous infection in the experiments of Mankiewicz and Liliak (78).

It is possible that at least a part of the enhancing effect in these experiments, with the limitations mentioned earlier was due to the endotoxin like substance of *C. albicans* or a similar substance of *C. Krusei*. A similar effect was illustrated by own experiments in which the survival time of mice injected with the soluble extract of *C. albicans* 2 hours before challenge with *Salmonella enteritidis* was shorter than in the controls (Figure 12).

As long however as the chemical nature of the own endotoxin like substance of *Candida* and of the various above mentioned extracts is unknown it is impossible to determine whether the chemically extracted preparations of Ruzicido et al. (120 121 122) and Yamabayashi (152) actually contained the same resistance-influencing substance which is present in the own soluble extract from *Candida*. The preparation of Mankiewicz and Liliak (78) was, similarly as the own soluble extract prepared from the supernatant remaining after disintegration of yeast cells, so that most of the cell wall material was apparently excluded from both preparations.

#### (u)(a) Effect of Double Infections with *Candida* and Another Organism on Nonspecific Resistance

When evaluating the following group of experiments it has to be realized that since the two organisms are living both may be capable of producing constantly an endotoxin like substance. They may however also produce antibiotic substances which may influence the development of the mixed infection.

Donomae and Kawamori (18) infected mice simultaneously with  $50 \times 10^6$  cells of *Candida albicans* and 1/10 of the minimal lethal dose of *Proteus vulgaris*. About 50 % of controls injected with *Candida* only were dead at the end of 7 days while all mice with the mixed infection succumbed in 3 days. Another group of mice was infected simultaneously with  $100 \times 10^6$  cells of *C. albicans* and 1/20 of the minimal lethal dose of *Proteus vulgaris*. About 20 % of controls injected with *Candida* only were dead after 7 days while all mice with the mixed infection died in 3 days. Similar results were obtained in a mixed infection with *C. albicans* and *Pseudomonas aeruginosa*. Death rates of mice injected simultaneously with *C. albicans* and 1/10 of the minimal lethal dose of either *Staphylococcus Streptococcus haemolyticus*, *Escherichia coli* or *Bacillus subtilis* did not differ from those of controls which received *C. albicans* only.

The enhancement in a simultaneous infection with *Candida* and *P. otus* or *Pseudomonas* was apparently due to liberation, by both *Candida* and the bacterial organisms, of endotoxin-like

peritoneally with  $5 \times 10^6$  cells of *Candida albicans*. Twenty four hours later intraperitoneal injections of  $10^5$  cells of *Saccharomyces cerevisiae* were started and repeated every other day for a total of 10 injections. When the mice were sacrificed 20 days after the injection of *Candida* it was found that the degree of dissemination of monillium was much more pronounced in animals treated with *Saccharomyces* than in untreated controls which received only *Candida*.

In another experiment Scherr studied the effect of one single injection of *Saccharomyces* containing the total number of living cells that were used in all 10 injections in the preceding experiment combined. Such an injection was given either 24 hours prior to the *Candida* infection together with it or 4, 48 or 72 hours later. While there was no essential difference between controls and treated mice when *Saccharomyces* was injected 24 hours before the infection, a markedly increased dissemination was noted in all other groups of mice, particularly in those that received yeast together with or 24 hours after the infection.

Hasenclever and Mitchell (40) injected mice intraperitoneally with either  $10^6$  cells of *Histoplasma capsulatum* or  $10^7$  cells of *Cryptococcus neoformans*  $4 \times 10^4$  spherule units of *Coccidioides immitis* or  $10^5$  conidia spores of *Aspergillus fumigatus* six days before they challenged them intracranially with  $10^7$  cells of *Candida albicans*. The life of pre-treated mice was somewhat prolonged as compared to mice which received *Candida* challenge only.

The experiments of Scherr (129) and

of Hasenclever and Mitchell (40) demonstrate that modification of the infection may take place when the two viable organisms in the experiment are fungi, pathogenic or not. The modifying influence may have been exerted by resistance-influencing substances of *Saccharomyces cerevisiae* *Histoplasma capsulatum* *Cryptococcus neoformans* *Coccidioides immitis* and *Aspergillus fumigatus*.

The experiments in this group demonstrate modifications in the course of infections in animals injected with two living bacterial or fungal microorganisms, one of which is *Candida*. The results show that in simultaneous infections with *Candida albicans* and *Proteus* or *Pseudomonas* the mortalities of mice are much higher than in infections with single organisms. When these bacteria were injected 2—24 hours after *Candida* the death rates were highest in mice injected with bacterial organisms 2 hours after *Candida*. The enhancing effect in these experiments was apparently due to the presence in both *Candida* and the bacterial organisms of endotoxin-like substances capable of reducing resistance to the injected organisms, which was demonstrated in own experiments (Figures 7, 8, 9 and 12). Experiments of others with dead organisms or their products described in Section (I) (a) and (c) are in general agreement with these results. No increase in death rates was found when gram-positive organisms were injected simultaneously with *Candida*. When *Escherichia coli* was used the increase in mortality rates was less pronounced. This may have been due to the production by

responsible for this protective activity against *Candida* especially since this influence was absent when heat killed cells of *E. coli* were used.

The finding of Gale and Sandoval (37) that *E. coli* given intraperitoneally 30 or 150 minutes after *Candida* protected some mice from death, but only when the dosage was  $10^6$  or  $10^7$  cells, appears to be in agreement with this idea. It is possible that with a lower dosage the antibiotic effect did not take place because of a too small number of *E. coli* capable of producing the hypothetic antibiotic substance, and with a higher dosage this antibiotic effect may have been nullified by the influence of the infection-enhancing effect of the endotoxin of *E. coli* which may have been ineffective at a lower dose.

In support of this concept is also the finding of the same investigators that *E. coli* inhibits *Candida* in vitro the experimental results of Baně (2) who found a substance in *E. coli* which exerted a fungistatic effect on *C. albicans* and the report of Freyschuss, Pehrson and Steenberg (35) who isolated coliforman an antibiotic from *E. coli* which is active against *C. albicans* and several other fungi. Similar work was also done by Wiedling (150) Paine (101) Rosebury Gale and Taylor (113) and Fischer (30) who observed inhibition of *C. albicans* by *E. coli*.

Mankiewicz and Luvak (78) inoculated 20 guinea pigs simultaneously with *Mycobacterium tuberculosis* and with 1 mg (wet weight) of *Candida albicans* by subcutaneous route. Twenty animals were inoculated with tubercle bacilli alone and kept as controls. An

additional control group of 20 guinea pigs was given 1 mg of *C. albicans* alone. Another group was inoculated with *M. tuberculosis* and a polysaccharide fraction of *C. albicans* (page 23)

Eleven of 20 guinea pigs inoculated with *M. tuberculosis* and *C. albicans* died within 6 weeks. During the same period of time 1 of 20 guinea pigs injected with *Candida* alone and 2 of 20 animals inoculated with *M. tuberculosis* alone died of unrelated bacterial infections.

On autopsies of surviving animals performed in the twelfth week after inoculation it was found that cultures of the liver spleen and lungs of guinea pigs inoculated with the two microorganisms were much more frequently positive for *M. tuberculosis* than the organs of guinea pigs inoculated with *M. tuberculosis* alone. *Candida* was isolated from regional lymph nodes but not from internal organs of guinea pigs with the simultaneous infection in the first 6 weeks. After this time *Candida* was not cultured from any tissue.

The shortened survival time resulting from a simultaneous infection with *C. albicans* and *M. tuberculosis* may have been due to the presence of a resistance influencing substance of *C. albicans* and possibly a similar substance in *M. tuberculosis* (127). It is also possible that the growth of *M. tuberculosis* may have been promoted by another mechanism (page 23) viz. an in vitro growth-promoting effect of the polysaccharide fraction of *C. albicans* as described by Mankiewicz, Stackiewicz and Luvak (79).

Scherr (129) injected mice intra

peritoneally with  $5 \times 10^6$  cells of *Candida albicans*. Twenty-four hours later intraperitoneal injections of  $10^5$  cells of *Saccharomyces cerevisiae* were started and repeated every other day for a total of 10 injections. When the mice were sacrificed 20 days after the injection of *Candida* it was found that the degree of dissemination of mycelia was much more pronounced in animals treated with *Saccharomyces* than in untreated controls which received only *Candida*.

In another experiment Scherr studied the effect of one single injection of *Saccharomyces* containing the total number of living cells that were used in all 10 injections in the preceding experiment combined. Such an injection was given either 24 hours prior to the *Candida* infection, together with it or 4, 48 or 72 hours later. While there was no essential difference between controls and treated mice when *Saccharomyces* was injected 24 hours before the infection, a markedly increased dissemination was noted in all other groups of mice particularly in those that received yeast together with or 24 hours after the infection.

Hasenclever and Mitchell (40) injected mice intraperitoneally with either  $10^6$  cells of *Histoplasma capsulatum* or  $10^5$  cells of *Cryptococcus neoformans*,  $4 \times 10^5$  sporeules of *Coccidioides immitis* or 10 conidia spores of *Aspergillus fumigatus* six days before they challenged them intravenously with  $10^5$  cells of *Candida albicans*. The life of pretreated mice was somewhat prolonged as compared to mice which received *Candida* challenge only.

The experiments of Scherr (129) and

of Hasenclever and Mitchell (40) demonstrate that modification of the infection may take place when the two viable organisms in the experiment are fungi, pathogenic or not. The modifying influence may have been exerted by resistance-influencing substances of *Saccharomyces cerevisiae*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Coccidioides immitis* and *Aspergillus fumigatus*.

The experiments in this group demonstrate modifications in the course of infections in animals injected with two living bacterial or fungal microorganisms, one of which is *Candida*. The results show that in simultaneous infections with *Candida albicans* and *Proteus* or *Pseudomonas* the mortalities of mice are much higher than in infections with single organisms. When these bacteria were injected 2–24 hours after *Candida* the death rates were highest in mice injected with bacterial organisms 2 hours after *Candida*. The enhancing effect in these experiments was apparently due to the presence in both *Candida* and the bacterial organisms of endotoxin-like substances capable of reducing resistance to the injected organisms, which was demonstrated in own experiments (Figures 7, 8, 11 and 12). Experiments of others with dead organisms or their products described in Section (1)(a) and (1)(b) are in general agreement with these results. No increase in death rates was found when gram-positive organisms were injected simultaneously with *Candida*. When *Escherichia coli* was used the increase in mortality rates was less pronounced. This may have been due to the production by

responsible for this protective activity against *Candida* especially since this influence was absent when heat killed cells of *E. coli* were used.

The finding of Gale and Sandoval (37) that *E. coli* given intraperitoneally 30 or 150 minutes after *Candida* protected some mice from death, but only when the dosage was  $10^8$  or  $10^7$  cells, appears to be in agreement with this idea. It is possible that with a lower dosage the antibiotic effect did not take place because of a too small number of *E. coli* capable of producing the hypothetic antibiotic substance, and with a higher dosage this antibiotic effect may have been nullified by the influence of the infection-enhancing effect of the endotoxin of *E. coli* which may have been ineffective at a lower dose.

In support of this concept is also the finding of the same investigators that *E. coli* inhibits *Candida* in vitro, the experimental results of Daniš (2) who found a substance in *E. coli* which exerted a fungistatic effect on *C. albicans* and the report of Freyschuss, Pehrson and Steenberg (35) who isolated coli formin an antibiotic from *E. coli* which is active against *C. albicans* and several other fungi. Similar work was also done by Wiedling (150) Paine (101) Rosebury, Gale and Taylor (113) and Fischer (30) who observed inhibition of *C. albicans* by *E. coli*.

Mankiewicz and Luvak (78) inoculated 20 guinea pigs simultaneously with *Mycobacterium tuberculosis* and with 1 mg (wet weight) of *Candida albicans* by subcutaneous route. Twenty animals were inoculated with tubercle bacilli alone and kept as controls. An

additional control group of 20 guinea pigs was given 1 mg of *C. albicans* alone. Another group was inoculated with *M. tuberculosis* and a polysaccharide fraction of *C. albicans* (page 23).

Eleven of 20 guinea pigs inoculated with *M. tuberculosis* and *C. albicans* died within 6 weeks. During the same period of time 1 of 20 guinea pigs injected with *Candida* alone and 2 of 20 animals inoculated with *M. tuberculosis* alone died of unrelated bacterial infections.

On autopsies of surviving animals performed in the twelfth week after inoculation it was found that cultures of the liver, spleen and lungs of guinea pigs inoculated with the two microorganisms were much more frequently positive for *M. tuberculosis* than the organs of guinea pigs inoculated with *M. tuberculosis* alone. *Candida* was isolated from regional lymph nodes but not from internal organs of guinea pigs with the simultaneous infection in the first 6 weeks. After this time *Candida* was not cultured from any tissue.

The shortened survival time resulting from a simultaneous infection with *C. albicans* and *M. tuberculosis* may have been due to the presence of a resistance-influencing substance of *C. albicans* and possibly a similar substance in *M. tuberculosis* (127). It is also possible that the growth of *M. tuberculosis* may have been promoted by another mechanism (page 23), viz. an in vitro growth-promoting effect of the polysaccharide fraction of *C. albicans* as described by Mankiewicz, Stachlewicz and Luvak (79).

Scherr (129) injected mice intra

peritoneally with  $5 \times 10^4$  cells of *Candida albicans*. Twenty-four hours later intraperitoneal injections of  $10^5$  cells of *Saccharomyces cerevisiae* were started and repeated every other day for a total of 10 injections. When the mice were sacrificed 20 days after the injection of *Candida* it was found that the degree of dissemination of moniliasis was much more pronounced in animals treated with *Saccharomyces* than in untreated controls which received only *Candida*.

In another experiment Scherr studied the effect of one single injection of *Saccharomyces* containing the total number of living cells that were used in all 10 injections in the preceding experiment combined. Such an injection was given either 24 hours prior to the *Candida* infection, together with it or 4, 48 or 72 hours later. While there was no essential difference between controls and treated mice when *Saccharomyces* was injected 24 hours before the infection, a markedly increased dissemination was noted in all other groups of mice, particularly in those that received yeast together with or 24 hours after the infection.

Haseenclever and Mitchell (40) injected mice intraperitoneally with either  $10^5$  cells of *Histoplasma capsulatum*,  $10^7$  cells of *Cryptococcus neoformans*,  $4 \times 10$  sporule units of *Coccidioides immitis* or  $10^7$  conidiospores of *Aspergillus fumigatus* six days before they challenged them intravenously with  $10^7$  cells of *Candida albicans*. The life of pretreated mice was somewhat prolonged as compared to mice which received *Candida* challenge only.

The experiments of Scherr (129) and

of Haseenclever and Mitchell (40) demonstrate that modification of the infection may take place when the two viable organisms in the experiment are fungi, pathogenic or not. The modifying influence may have been exerted by resistance-influencing substances of *Saccharomyces cerevisiae*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Coccidioides immitis* and *Aspergillus fumigatus*.

The experiments in this group demonstrate modifications in the course of infections in animals injected with two living bacterial or fungal microorganisms, one of which is *Candida*. The results show that in simultaneous infections with *Candida albicans* and *Proteus* or *Pseudomonas* the mortalities of mice are much higher than in infections with single organisms. When these bacteria were injected 2–24 hours after *Candida* the death rates were highest in mice injected with bacterial organisms 2 hours after *Candida*. The enhancing effect in these experiments was apparently due to the presence in both *Candida* and the bacterial organisms of endotoxin-like substances capable of reducing resistance to the injected organisms, which was demonstrated in own experiments (Figures 7, 8, 9 and 12). Experiments of others with dead organisms or their products described in Section 11(a) and 11(c), are in general agreement with these results. No increase in death rates was found when gram-positive organisms were injected simultaneously with *Candida*. When *Escherichia coli* was used the increase in mortality rates was less pronounced. This may have been due to the production by

responsible for this protective activity against *Candida* especially since this influence was absent when heat killed cells of *E. coli* were used.

The finding of Gale and Sandoval (37) that *E. coli* given intraperitoneally 30 or 150 minutes after *Candida* protected some mice from death, but only when the dosage was  $10^8$  or  $10^7$  cells, appears to be in agreement with this idea. It is possible that with a lower dosage the antibiotic effect did not take place because of a too small number of *E. coli* capable of producing the hypothetical antibiotic substance, and with a higher dosage this antibiotic effect may have been nullified by the influence of the infection-enhancing effect of the endotoxin of *E. coli* which may have been ineffective at a lower dose.

In support of this concept is also the finding of the same investigators that *E. coli* inhibits *Candida* in vitro, the experimental results of Banič (2) who found a substance in *E. coli* which exerted a fungistatic effect on *C. albicans* and the report of Freyschuss, Pehrson and Steenberg (35) who isolated coliformin an antibiotic from *E. coli* which is active against *C. albicans* and several other fungi. Similar work was also done by Wiedling (150) Paine (101) Rosebury Gale and Taylor (113) and Fischer (30) who observed inhibition of *C. albicans* by *E. coli*.

Mankiewicz and Llivak (78) inoculated 20 guinea pigs simultaneously with *Mycobacterium tuberculosis* and with 1 mg (wet weight) of *Candida albicans* by subcutaneous route. Twenty animals were inoculated with tubercle bacilli alone and kept as controls. An

additional control group of 20 guinea pigs was given 1 mg of *C. albicans* alone. Another group was inoculated with *M. tuberculosis* and a polysaccharide fraction of *C. albicans* (page 23)

Eleven of 20 guinea pigs inoculated with *M. tuberculosis* and *C. albicans* died within 6 weeks. During the same period of time 1 of 20 guinea pigs injected with *Candida* alone and 2 of 20 animals inoculated with *M. tuberculosis* alone died of unrelated bacterial infections.

On autopsies of surviving animals performed in the twelfth week after inoculation it was found that cultures of the liver spleen and lungs of guinea pigs inoculated with the two microorganisms were much more frequently positive for *M. tuberculosis* than the organs of guinea pigs inoculated with *M. tuberculosis* alone. *Candida* was isolated from regional lymph nodes but not from internal organs of guinea pigs with the simultaneous infection in the first 6 weeks. After this time *Candida* was not cultured from any tissue.

The shortened survival time resulting from a simultaneous infection with *C. albicans* and *M. tuberculosis* may have been due to the presence of a resistance-influencing substance of *C. albicans* and possibly a similar substance in *M. tuberculosis* (127). It is also possible that the growth of *M. tuberculosis* may have been promoted by another mechanism (page 23) viz. an in vitro growth promoting effect of the polysaccharide fraction of *C. albicans* as described by Mankiewicz, Stackiewicz and Llivak (79)

Scherr (129) injected mice intra

days. By the time the second injection was administered in the experiment in which the interval between the two injections was 30 days the primary infectious process may have been subsiding and there was apparently not enough of the endotoxin-like substance produced in some animals to counteract the protective effect of the specific antibodies which may have developed in the meantime. The 2/3 that did die may have been rendered hypersensitive, their antibodies may not have been sufficient and the endotoxin-like substance may have stimulated the spread of the organisms in the second injection.

Fischer and Horbach (31) have pretreated 30 mice with 0.5 cc. of a 0.1 % suspension of living cells of *Candida albicans* (about 6,250,000 cells) intraperitoneally twenty-four hours before they challenged them by the same route with 0.2 cc. of a 4 % suspension of living cells of the same fungus (about 100,000,000 cells). Nineteen mice died in 3 weeks. Among 30 controls which received the challenge dose only there were 15 deaths after 3 weeks. Of 15 controls receiving pretreatment only and no challenge 4 mice died.

In this experiment pretreatment with small number of cells was given 24 hours before a sizeable challenge. The number of mice dying after the two injections was slightly higher than that of control mice receiving challenge only. A possible explanation of this relatively slight infection-enhancing effect may be that the time between the injections was rather short to afford sufficient multiplication of the fungus which would liberate enough endotoxin-like

substance which would enhance the growth of fungi contained in the challenge dose.

Hasenclever and Mitchell (40, 41) injected mice intraperitoneally with  $10^7$  cells of *Candida albicans* and challenged them 6 days later with the same number of cells intravenously. They found that 80 % of the controls which received challenge only were dead 17 hours after the injection whereas only 10 % of the pretreated mice had succumbed during this period. All controls were dead at the end of 24 hours while all pretreated mice were dead at 72 hours. In experiments in which the interval between pretreatment and challenge was 1, 3, 10 or 14 days there was no appreciable change in mortality rates of mice. When the challenge dose was increased to  $5 \times 10^7$  cells in mice pretreated with  $10^7$  viable cells intraperitoneally there was no significant difference between pretreated mice and controls. No effect on the survival times of mice was caused by pretreatment of mice with  $2 \times 10^7$  of viable cells of *C. albicans* given intraperitoneally 7 and again 4 days before challenge.

Pretreatment with living cells of members of the genus *Candida* other than *albicans* (*C. stellatoidea*, *C. tropicalis*, *C. Guilliermondii*, *C. parapsilosis*) increased the survival time of mice challenged with *C. albicans* similarly as pretreatment with *C. albicans* but to a lesser extent.

The delayed time of death in mice pretreated intraperitoneally with living cells of *C. albicans* 6 days prior to intravenous challenge with the same organism may be due to the fact that the infec-



*E. coli* of an antibiotic substance active against *C. albicans* in vitro which was reported by several authors.

Experiments with a simultaneous infection with *C. albicans* and *Mycobacterium tuberculosis* resulted in an enhancement of the tuberculous infection. This may have been based on the combination of the same mechanism as the enhancement in the simultaneous infections described above, and the promoting effect of the polysaccharide fractions of *C. albicans* effective in vitro on *M. tuberculosis* which was described by Mankiewicz, Stackiewicz and Luvak (79).

Experiments with fungal organisms, pathogenic or not injected into mice infected with *C. albicans* led to similar results. A single or repeated administration of *Saccharomyces cerevisiae* to mice after they had been infected with *C. albicans* or a simultaneous injection of both organisms led to an increased dissemination of *Candida*. The life of mice injected with *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Coccidioides immitis* or *Aspergillus fumigatus* 6 days before challenge with *C. albicans* was somewhat prolonged. It is possible that the modification of the *Candida* infection may have been due to the presence in the mentioned fungi, of endotoxin-like substances.

(11)(b) *Effects of Living Candida on Nonspecific Resistance to a Candida Infection*

Ninni and Fittipaldi (96) used a pathogenic species of *Candida* which they called *Candida Ninni-Fittipaldi*. They

noted that guinea pigs injected intraperitoneally, with one large dose of living cells of this *Candida* containing about 1 billion cells do not die. When they are injected, however with four small doses of 50 000 000 cells every four days, about one third of them die in 1—2 days after the fourth injection. There were no survivors after the eighth injection among 12 guinea pigs given 50 000 000 cells intraperitoneally, every other day. When injected intraperitoneally with 50,000,000 cells every 4—5 days four times and challenged with 500 000 000 cells 5—10 days after the last injection 7 of 8 guinea pigs died 1—3 days after challenge.

The experiments in which guinea pigs died after several intraperitoneal doses of *Candida* the total of which would be sublethal if given in one injection, may be based on liberation of the endotoxin-like substance from living *Candida* which stimulates the growth of the fungus in every subsequent injection as long as the infection has not subsided and the susceptible tissues are still being supplied with enough of this substance. The experiment in which most animals died after they had been injected 4 times with a small dose and reinjected 5—10 days after the last injection may be due to the same mechanism or possibly due to sensitization.

Ninni and Fittipaldi have also shown in another experiment which is reviewed in the chapter on specific immunity (pages 37—38) that all guinea pigs receiving two intraperitoneal injections of 1 000 000 000 cells each given 10 days apart died while only 2/3 of them succumbed when the interval was 30

the development of specific antibodies.

Another experiment showed that the life of mice was prolonged when an injection of *C. albicans* or another species of *Candida* was followed 5 days later by a challenge with *C. albicans*. A high concentration of the endotoxin-like substance may have been present in the tissues several days before challenge so that the resulting effect was an increase in resistance.

### (B) Allergy and its Relation to Immunity in Moniliasis

The study of the relation between allergy and susceptibility or resistance to *Candida* infections is of interest because of basic considerations concerning the pathogenesis of *Candida* infections (100) particularly in view of the experimental findings that suggest the possibility that allergy may be one of the mechanisms for the establishment of disseminated moniliasis. The question of this relationship is also important because allergic reactions may interfere with the ultimate outcome of immunization since the antigen used for immunization may also contain an allergen. The elimination of such an allergen may thus lead to the production of a more effective vaccine.

#### (1) Allergy to Produce Bronchopulmonary Moniliasis without Sensitization

Loe, Kurotchkin and Wu (76) studied the question whether bronchopulmonary moniliasis may be produced in healthy animals by inoculation of the fungus intratracheally or what factors may be necessary for its establishment. They were unable to produce bronchopul-

monary moniliasis in rabbits by intratracheal injections of *Monilia tropicalis* given twice at three day intervals. They were equally unable to produce the disease when direct mechanical injury to the lung was brought about by the injection of either glass powder charcoal or phenol and was followed by intratracheal inoculation of *Monilia tropicalis*. Intravenous injections of small doses of liquid paraffin, though causing the formation of small embolic foci in the lungs, did not enable the *Monilia tropicalis* to develop active lesions. Intravenous injections of chaulmoogra oil produced stable embolic lesions in which *Monilia tropicalis* injected through the trachea developed and gave rise to localized pulmonary or generalized infection.

Reimann and Kurotchkin (108) were unable to produce any pulmonary lesions in monkeys by similar methods as those used in the mentioned experiments in rabbits. Intravenous administration of chaulmoogra oil in monkeys did not predispose to the formation of monilial lesions.

#### (b) Production of Active and Passive Anaphylaxis with *Candida* or its Products

In the next series of experiments Kurotchkin and Lim (66) tried to solve the question whether allergic sensitization may be produced by *Candida*. They injected guinea pigs intraperitoneally with heat-killed cells of *Monilia puloni*, *M. Pinoyi* and *Saccharomyces cerevisiae*. Each dose was equal to 1/10 of an agar slant culture. The animals received 1 to 3 such injections. At the end of two weeks all guinea pigs were injected with

tion produced by the first injection may have been subsiding by the time the second injection was given. A sufficient amount of the endotoxin like substance may have been present in the susceptible tissues of the mice several days prior to challenge and very little of it at the time of the challenge. As was shown by own experiments with the soluble extract of *C. albicans* (Figures 2, 3 4 and 5) and by those of Hasenclever and Mitchell (40) mentioned on page 21 in which dead cells of *Candida* were used as pre-treatment the endotoxin-like substance or dead cells injected several days before challenge enhance the resistance to it.

The experiments of Hasenclever and Mitchell (40) with viable cells also suggest that an endotoxin like substance similar to that of *C. albicans* is probably present in members of the genus *Candida* other than *albicans* as may also be concluded from the experiments of Rzuclido et al. (120) which are reviewed on page 22

The results of these experiments which show the effects of variously spaced repeated injections of living cells of *Candida* given within a relatively short time cannot be easily predicted because of the variables inherent in the use of multiplying agents. The size of the inoculum is of major importance here, since the spread of the fungus in the animal the duration and time of maximum liberation of the endotoxin like substance and consequently either enhancement or depression of resistance to challenge largely depend on it.

It appears that these experiments should be evaluated mainly on the basis

of two factors, both of which are besides timing of the injections, largely influenced by the size of the inoculum. One of them is the presence or absence of a high level of the endotoxin-like substance in the animal tissues at the time of challenge. If a high level is present the infection caused by the challenge will be enhanced (Figure 9) The other factor is the presence or absence of this substance in the tissues several days before challenge. If present it will enhance resistance to the infection (Figures 2 3 4 and 5) Various combinations of these two factors may naturally cause enhancement depression or no effect on the resistance of the animal.

Four to eight small inoculations of *C. albicans* repeated every 2-4 days killed the majority of guinea pigs while one large injection containing more yeast cells than the total of the repeated injections did not. Liberation of the endotoxin-like substance a high level of which may have been present at the time of each subsequent injection may have stimulated the growth of the fungus. The situation may have been similar to that in the own experiment depicted in Figure 9

Two large injections of *C. albicans* given ten days apart killed all guinea pigs while two identical large injections given 30 days apart killed only two thirds of them. When the interval was 10 days enough endotoxin-like substance may have been present at the time of challenge to enhance it. When the interval was 30 days the effect of this substance may have disappeared by the time of the second injection and some animals may have been protected due to

the development of specific antibodies.

Another experiment showed that the life of mice was prolonged when an injection of *C. albicans* or another species of *Candida* was followed 6 days later by a challenge with *C. albicans*. A high concentration of the endotoxin-like substance may have been present in the throat several days before challenge so that the resulting effect was an increase in resistance.

### (B) Allergy and its Relation to Immunity in Moniliasis

The study of the relation between allergy and susceptibility or resistance to *Candida* infections is of interest because of basic considerations concerning the pathogenesis of *Candida* infections (100) particularly in view of the experimental findings that suggest the possibility that allergy may be one of the mechanisms for the establishment of disseminated moniliasis. The question of this relationship is also important because allergic reactions may interfere with the ultimate outcome of immunization since the antigen used for immunization may also contain an allergen. The elimination of such an allergen may thus lead to the production of a more effective vaccine.

#### (a) Allergy in Producing Bronchopulmonary Moniliasis without Sensitization

Linn, Kurotschkin and Wu (76) studied the question whether bronchopulmonary moniliasis may be produced in healthy animals by inoculations of the fungus intratracheally or what factors may be necessary for its establishment. They were unable to produce bronchopul-

monary moniliasis in rabbits by intratracheal injections of *Monilia tropicalis* given twice at three day intervals. They were equally unable to produce the disease when direct mechanical injury to the lung was brought about by the injection of either glass powder charcoal or phenol and was followed by intratracheal inoculation of *Monilia tropicalis*. Intravenous injections of small doses of liquid paraffin, though causing the formation of small embolic foci in the lungs, did not enable the *Monilia tropicalis* to develop active lesions. Intravenous injections of chaulmoogra oil produced stable embolic lesions in which *Monilia tropicalis* injected through the trachea developed and gave rise to localized pulmonary or generalized infection.

Reimann and Kurotschkin (108) were unable to produce any pulmonary lesions in monkeys by similar methods as those used in the mentioned experiments in rabbits. Intravenous administration of chaulmoogra oil in monkeys did not predispose to the formation of monilial lesions.

#### (b) Production of Active and Passive Anaphylaxis with *Candida* or its Products

In the next series of experiments Kurotschkin and Linn (66) tried to solve the question whether allergic sensitization may be produced by *Candida*. They injected guinea pigs intraperitoneally with heat-killed cells of *Monilia pilosus* Af Plesny and *Saccharomyces cerevisiae*. Each dose was equal to 1/10 of an agar slant culture. The animals received 1 to 3 such injections. At the end of two weeks all guinea pigs were injected with

tion produced by the first injection may have been subsiding by the time the second injection was given. A sufficient amount of the endotoxin-like substance may have been present in the susceptible tissues of the mice several days prior to challenge, and very little of it at the time of the challenge. As was shown by own experiments with the soluble extract of *C. albicans* (Figures 2 3 4 and 5) and by those of Hasenclever and Mitchell (40) mentioned on page 21 in which dead cells of *Candida* were used as pre-treatment, the endotoxin-like substance or dead cells injected several days before challenge enhance the resistance to it.

The experiments of Hasenclever and Mitchell (40) with viable cells also suggest that an endotoxin like substance similar to that of *C. albicans* is probably present in members of the genus *Candida* other than *albicans* as may also be concluded from the experiments of Rucidlo et al (120) which are reviewed on page 22.

The results of these experiments which show the effects of variously spaced repeated injections of living cells of *Candida* given within a relatively short time cannot be easily predicted because of the variables inherent in the use of multiplying agents. The size of the inoculum is of major importance here, since the spread of the fungus in the animal, the duration and time of maximum liberation of the endotoxin-like substance and consequently either enhancement or depression of resistance to challenge largely depend on it.

It appears that these experiments should be evaluated mainly on the basis

of two factors, both of which are, besides timing of the injections, largely influenced by the size of the inoculum. One of them is the presence or absence of a high level of the endotoxin-like substance in the animal tissues at the time of challenge. If a high level is present the infection caused by the challenge will be enhanced (Figure 9) The other factor is the presence or absence of this substance in the tissues several days before challenge. If present it will enhance resistance to the infection (Figures 2, 3 4 and 5) Various combinations of these two factors may naturally cause enhancement, depression or no effect on the resistance of the animal.

Four to eight small inoculations of *C. albicans* repeated every 2-4 days killed the majority of guinea pigs while one large injection containing more yeast cells than the total of the repeated injections did not. Liberation of the endotoxin-like substance a high level of which may have been present at the time of each subsequent injection may have stimulated the growth of the fungus. The situation may have been similar to that in the own experiment depicted in Figure 9

Two large injections of *C. albicans* given ten days apart killed all guinea pigs while two identical large injections given 30 days apart killed only two thirds of them. When the interval was 10 days enough endotoxin-like substance may have been present at the time of challenge to enhance it. When the interval was 30 days the effect of this substance may have disappeared by the time of the second injection and some animals may have been protected due to

the development of specific antibodies.

Another experiment showed that the life of mice was prolonged when an injection of *C. albicans* or another species of *Candida* was followed 6 days later by a challenge with *C. albicans*. A high concentration of the endotoxin-like substance may have been present in the tissues several days before challenge so that the resulting effect was an increase in resistance.

### (B) Allergy and its Relation to Immunity in Monilliasis

The study of the relation between allergy and susceptibility or resistance to *Candida* infections is of interest because of basic considerations concerning the pathogenesis of *Candida* infections (100) particularly in view of the experimental findings that suggest the possibility that allergy may be one of the mechanisms for the establishment of disseminated monilliasis. The question of this relationship is also important because allergic reactions may interfere with the ultimate outcome of immunization since the antigen used for immunization may also contain an allergen. The elimination of such an allergen may thus lead to the production of a more effective vaccine.

#### (a) Attempts to Produce Bronchopulmonary Monilliasis without Sensitization

Lim, Kurotchkin and Wu (76) studied the question whether bronchopulmonary monilliasis may be produced in healthy animals by inoculations of the fungus intratracheally or what factors may be necessary for its establishment. They were unable to produce bronchopul-

monary monilliasis in rabbits by intra tracheal injections of *Af. nil. a. tropicalis* given twice at three day intervals. They were equally unable to produce the disease when direct mechanical injury to the lung was brought about by the injection of either glass powder, charcoal or phenol and was followed by intra tracheal inoculation of *Monilia tropicalis*. Intravenous injections of small doses of liquid paraffin, though causing the formation of small embolic foci in the lungs, did not enable the *Monilia tropicalis* to develop active lesions. Intravenous injections of chaulmoogra oil produced stable embolic lesions in which *Monilia tropicalis* injected through the trachea developed and gave rise to localized pulmonary or generalized infection.

Reimann and Kurotchkin (108) were unable to produce any pulmonary lesions in monkeys by similar methods as those used in the mentioned experiments in rabbits. Intravenous administration of chaulmoogra oil in monkey did not predispose to the formation of monilial lesions.

#### (b) Production of Active and Passive Anaphylaxis with *Candida* or its Products

In the next series of experiments Kurotchkin and Lim (66) tried to solve the question whether allergic sensitization may be produced by *Candida*. They injected guinea pigs intraperitoneally with heat-killed cells of *Monilia pul. as Af. Pruyi* and *Saccharomyces cerevisiae*. Each dose was equal to 1/10 of an agar slant culture. The animals received 1 to 3 such injections. At the end of two weeks all guinea pigs were injected with

0.5 to 4.0 mg of a homologous water soluble substance derived from one of the mentioned fungi. Both *Monilia* cultures produced active sensitization as evidenced by a typical anaphylactic shock following an injection of the *Monilia* extract. Sensitization was not produced by injections of heat killed cells of *Saccharomyces*. It was also found that heat killed *M. pilosus* grown in glucose broth sensitized more readily than the same fungus grown on Sabouraud's agar. The authors believed that the sensitizing power of the mycelial growth which was mostly found in broth was distinctly higher than that of the yeast cell growth of the same fungus.

Kurotchkin and Lim (67) found that incubation of cells of *Monilia Pinoyi* with formaldehyde decreased the sensitizing power of the fungus in guinea pigs.

Lim and Kurotchkin (74) have examined the relationship between the production of anaphylaxis and formation of precipitin and complement fixing antibodies in guinea pigs injected with heat killed cultures of *Monilia Pinoyi* and *M. pilosus*. They found that in the case of *M. Pinoyi* sensitization appeared before the precipitin or complement fixing antibodies could be demonstrated, while with *M. pilosus* anaphylactic response was produced only in the presence of demonstrable precipitina. They also reported that the precipitin reaction was more sensitive than complement fixation.

Lim and Kurotchkin (75) studied the time factor in anaphylactic sensitization with heat killed cultures of *Monilia tropicalis*. They noted that fatal anaphylactic shock could be produced in

guinea pigs when a soluble carbohydrate from *Monilia* was injected as early as six days after the animals had been sensitized. This period was shorter than that usually observed in serum sensitization. The authors have also found that with smaller or larger than optimum sensitizing doses a longer time (15 days) was required. No anaphylaxis occurred following challenging injections containing either very high or very low amounts of the carbohydrate.

Hesten and Mott (61) used similar methods to study hypersensitivity to water soluble substances prepared by chemical extraction of whole cells of *Monilia albicans*, *M. pilosus*, *M. para pilosus*, *Willia anomala* and *Saccharomyces cerevisiae*. Injections of 10 mg of the specific substance from *Monilia pilosus* on 10 consecutive days failed to produce an anaphylactic shock in guinea pigs injected 21 days after the last sensitizing injection with 0.25 to 4 mg of the same soluble substance. Active sensitization was successful when guinea pigs were given 10 consecutive daily intraperitoneal injections of 0.4 cc. of a 5% suspension of heat killed washed cells of *Monilia pilosus*. Seven out of eight animals given an intravenous shocking dose of 0.5 to 2.0 mg of the soluble substance 21 days after the last injection developed an anaphylactic shock and two of them died. Two guinea pigs received 0.2 cc. of a 20% suspension of ground *Monilia pilosus* and one animal was given 0.4 cc. of a 10% suspension of intact living cells of *M. pilosus* as a shocking dose instead of the soluble substance. All three developed an anaphylactic shock and one of them died.

Passive anaphylaxis to the soluble substance of *M. pilosus* was produced by an intravenous injection of 0.5 or 1.0 cc. of serum from a rabbit immunized against *M. pilosus*. Twenty-four hours later each of 8 guinea pigs received 0.01 to 1.0 mg. of the homologous soluble substance intravenously. Six of the eight passively sensitized guinea pigs died in anaphylactic shock, the remaining two survived but showed signs of shock. Animals sensitized with each anti-serum were also challenged 24 hours later with soluble substances prepared from each of 4 other aforementioned fungi. The heterologous soluble substances often induced anaphylactic shock in passively sensitized guinea pigs. A close relationship was found between positive precipitation reactions and the production of anaphylaxis.

(c) Production of Bronchopulmonary Moniliasis in Sensitized Animals

After they had failed to demonstrate a production of bronchopulmonary moniliasis in healthy animals and in most of those with a mechanical injury to the lung, and after they had produced sensitization to *Candida* as evidenced by anaphylactic shock, Kurotchkin and Lam (68) attempted to produce bronchomoniliasis in normal and passively or actively sensitized rabbits. They injected 5 animals intratracheally on 2 successive occasions, 7 days apart, with 1/5 of an agar culture of *Monilia tropicalis*. Rabbits were killed 2 weeks later and no lesions were found on autopsy. A second group of 5 rabbits received an intratracheal injection of 1 cc. of an anti-*Monilia* serum followed by 2 intra-

tracheal injections of *Monilia tropicalis* 7 days apart. The results were essentially negative except for one rabbit in which a monilial lesion was found in one lung. A third group of 8 rabbits received 3 intravenous injections of heat-killed cultures of *Monilia tropicalis* at 5-day intervals. Ten days after the last injection, when precipitation tests became positive, the rabbits were inoculated intratracheally with *M. tropicalis*. This injection was repeated 7 days later. Two weeks after the second inoculation autopsies revealed that 6 of 8 animals developed localized pulmonary lesions which were nodular caseous in the center. Pre-disposing sensitivity was thought to account for the pathologic lesions.

Similar experiments were done by Vogel and Krehl (145) who used a single injection of dead *Candida* and adjuvant or living *Candida* for sensitization and living or dead *Candida* for intratracheal challenge. These authors injected two groups of nine and one group of five guinea pigs subcutaneously with a water-in-oil emulsion containing 0.22 cc. of a "thick saline suspension" of heat-killed *Candida albicans* and an adjuvant containing 3 mg. of autoclaved tubercle bacilli in mineral oil and lanolin. One group of 9 guinea pigs was injected at the same time with a single injection of a "heavy suspension" of living cells of *C. albicans*. Another group of 9 guinea pigs was not pretreated in any way.

Animals in one of the 3 groups which received dead *Candida* with adjuvant, animals of the group which was given living *Candida* and the guinea pigs without any pretreatment, were given



an intratracheal injection of either  $5 \times 10^8$ ,  $2.3 \times 10^7$  or  $1.2 \times 10^6$  of living cells of *C. albicans* 12 days after the pretreatment. The group of 5 animals pretreated with dead *Candida* and adjuvant received a challenge of  $5 \times 10^8$  of dead cells of *Candida*. The third group pretreated with dead *Candida* and adjuvant received no challenge.

While pretreated guinea pigs, especially those that received dead *Candida* with adjuvant, became highly sensitive to skin test materials, it was impossible to induce a chronic or lethal infection by intratracheal challenge with living organisms. The animals were sacrificed nine days after challenge. Hemorrhagic changes in the lungs and thickening of bronchial walls and alveolar septa seen in guinea pigs pretreated with the dead fungus and adjuvant and challenged with living *Candida* and to a lesser extent in those challenged with dead *Candida* were viewed as an expression of hypersensitivity developing in the lung tissue.

The experiments of the group of Kurotchkin, Linn, Remann and Wu (66—68 74—76 108) show that bronchopulmonary moniliasis cannot be produced in healthy rabbits or monkeys by intratracheal inoculations of the fungus. These investigators were equally unable to induce bronchopulmonary moniliasis by mechanical injury to the lung followed by intratracheal inoculation of *Candida*. They did, however, produce pulmonary lesions due to *Candida* in rabbits actively sensitized with heat killed cells of *Candida*. They concluded from these experiments that allergy is one of the possible mechanisms

for the establishment of disseminated moniliasis.

Kesten and Mott (61) produced hypersensitivity to dead cells of *Candida* but not to a soluble substance of the organism. They also succeeded in sensitizing rabbits passively. Vogel and Krehl (145) produced hemorrhagic changes in the lungs and thickening of bronchial walls and alveolar septa by sensitizing guinea pigs. Own experiments demonstrate that it is possible to produce anaphylaxis regularly with a fraction of the organism, viz. the soluble extract prepared by the disintegration of *Candida*, while previous authors were able to produce it with whole cells only. The fraction used unsuccessfully by Kesten and Mott for the production of anaphylaxis was prepared by chemical extraction of whole intact cells of *Candida* so that it appears that it contained predominantly material originating on or close to the cell surface, or that at least the content of materials of cytoplasmic origin was much lower than in the own soluble extract which was prepared by disintegration of the yeast cells and contained mainly the cytoplasm, besides, possibly the slimy layer covering the outer surface of the cell wall, and some soluble constituents of the cell walls (pages 53—54). It was impossible under my experimental conditions, to produce anaphylaxis in guinea pigs using cell walls for sensitization. All these facts point to the assumption that most of the sensitizing properties reside in the cytoplasm.

It appears, therefore, that since the soluble extract used in my experiments does not contain a specific protective

antigen, since it contains a highly sensitizing substance and since it also contains the endotoxin-like substance capable, under certain conditions, of counteracting the effect of specific immunization, it should not be a part of a protective vaccine against *Candida*.

### (C) Specific Immunity

#### (i) Active Immunization

Among the various experiments with the aim of inducing immunity against monobas in animals the attempts at active immunization by the introduction of variously prepared antigens are the most numerous. It is impossible to evaluate a great many of them since some reports do not contain enough information concerning the number of organisms used, the timing of injections and the interval between the last vaccine injection and challenge.

##### (a) Active Immunization with Living Cells in Rabbits

Rabbits were used by Ostrowsky (99) who reported preliminary results of his experiments which indicated that increased resistance could be induced by repeated subcutaneous injections of the living but attenuated fungus.

Roger (111 and 112) was able to immunize rabbits by injecting intravenously increasing doses of an *Oidium albicans* culture of low virulence. These rabbits were able to survive a challenge dose of two to three lethal doses. Details concerning the number of organisms or timing of injections were not given.

Roger also observed abundant growth of *Oidium albicans* in normal rabbit serum as compared to a very marked inhibition of growth in the serum of immunized animals. He noticed agglutination of *Candida* cells in the serum of immunized rabbits and the presence of a thick hyaline membrane surrounding the yeast cells. This membrane was sometimes lightly striated, with poorly defined borders. Its thickness was 5 to 10 times that of a normal cell wall.

Charnin and Ostrowsky (11) reported immunization of rabbits with repeated intravenous injections of a living but attenuated *Oidium albicans*. The fungus did not grow well in the serum of immunized animals. Details of the experiments are not given.

Concetti (14) reported that rabbits pretreated with repeated increasing intravenous doses of cultures of *Oidium albicans* subjected to slow desiccation for 1 or 2 weeks survived an intravenous challenge with a number of organisms much higher than the lethal dose. Neither the exact number of organisms used for immunization or challenge nor the timing of the injections were given.

van der Lenden (145) administered to rabbits six subcutaneous injections of increasing amounts of live or heat-killed organisms of *Monilia albicans*. The injections were given every eight days. After an unspecified length of time the animals were challenged with an intravenous injection of 2.5 times the lethal dose for the same organism. All rabbits died in less than four days presenting typical symptoms of generalized moniliasis.

The information given in all of the

an intratracheal injection of either  $5 \times 10^5$ ,  $2.3 \times 10^7$  or  $1.2 \times 10^8$  of living cells of *C. albicans* 12 days after the pretreatment. The group of 5 animals pretreated with dead *Candida* and adjuvant received a challenge of  $5 \times 10^8$  of dead cells of *Candida*. The third group pretreated with dead *Candida* and adjuvant received no challenge.

While pretreated guinea pigs, especially those that received dead *Candida* with adjuvant, became highly sensitive to skin test materials, it was impossible to induce a chronic or lethal infection by intratracheal challenge with living organisms. The animals were sacrificed nine days after challenge. Hemorrhagic changes in the lungs and thickening of bronchial walls and alveolar septa seen in guinea pigs pretreated with the dead fungus and adjuvant and challenged with living *Candida* and to a lesser extent in those challenged with dead *Candida* were viewed as an expression of hypersensitivity developing in the lung tissue.

The experiments of the group of Kurotchkin, Lim, Remann and Wu (66-68, 74-76, 108) show that bronchopulmonary moniliasis cannot be produced in healthy rabbits or monkeys by intratracheal inoculations of the fungus. These investigators were equally unable to induce bronchopulmonary moniliasis by mechanical injury to the lung followed by intratracheal inoculation of *Candida*. They did, however, produce pulmonary lesions due to *Candida* in rabbits actively sensitized with heat-killed cells of *Candida*. They concluded from these experiments that allergy is one of the possible mechanisms

for the establishment of disseminated moniliasis.

Keaten and Mott (61) produced hypersensitivity to dead cells of *Candida* but not to a soluble substance of the organism. They also succeeded in sensitizing rabbits passively Vogel and Krehl (145) produced hemorrhagic changes in the lungs and thickening of bronchial walls and alveolar septa by sensitizing guinea pigs. Own experiments demonstrate that it is possible to produce anaphylaxis regularly with a fraction of the organism, viz. the soluble extract prepared by the disintegration of *Candida* while previous authors were able to produce it with whole cells only. The fraction used unsuccessfully by Keaten and Mott for the production of anaphylaxis was prepared by chemical extraction of whole intact cells of *Candida* so that it appears that it contained predominantly material originating on or close to the cell surface or that at least the content of materials of cytoplasmic origin was much lower than in the own soluble extract which was prepared by disintegration of the yeast cells and contained mainly the cytoplasm, besides, possibly the slimy layer covering the outer surface of the cell wall, and some soluble constituents of the cell walls (pages 53-54). It was impossible under my experimental conditions, to produce anaphylaxis in guinea pigs using cell walls for sensitization. All these facts point to the assumption that most of the sensitizing properties reside in the cytoplasm.

It appears, therefore, that since the soluble extract used in my experiments does not contain a specific protective

antigen, since it contains a highly sensitizing substance and since it also contains the endotoxin-like substance capable, under certain conditions, of counteracting the effect of specific immunization, it should not be a part of a protective vaccine against *Candida*.

### (C) Specific Immunity

#### (i) Active Immunization

Among the various experiments with the aim of inducing immunity against moniliasis in animals the attempts at active immunization by the introduction of variously prepared antigens are the most numerous. It is impossible to evaluate a great many of them since some reports do not contain enough information concerning the number of organisms used, the timing of injections and the interval between the last vaccine injection and challenge.

#### (a) Active Immunization with Living Cells in Rabbits

Rabbits were used by Ostrowsky (99) who reported preliminary results of his experiments which indicated that increased resistance could be induced by repeated subcutaneous injections of the living but attenuated fungus.

Roger (111 and 112) was able to immunize rabbits by injecting intravenously increasing doses of an *Oidium albicans* culture of low virulence. These rabbits were able to survive a challenge dose of 10 to three lethal doses. Details concerning the number of organisms or timing of injections were not given.

Roger also observed abundant growth of *Oidium albicans* in normal rabbit serum as compared to a very marked inhibition of growth in the serum of immunized animals. He noticed agglutination of *Candida* cells in the serum of immunized rabbits and the presence of a thick hyaline membrane surrounding the yeast cells. This membrane was sometimes lightly striated, with poorly defined borders. Its thickness was 5 to 10 times that of a normal cell wall.

Charrin and Ostrowsky (11) reported immunization of rabbits with repeated intravenous injections of a living but attenuated *Oidium albicans*. The fungus did not grow well in the serum of immunized animals. Details of the experiments are not given.

Concetti (14) reported that rabbits pretreated with repeated increasing intravenous doses of cultures of *Oidium albicans* subjected to slow desiccation for 1 or 2 weeks survived an intravenous challenge with a number of organisms much higher than the lethal dose. Neither the exact number of organisms used for immunization or challenge nor the timing of the injections were given.

van der Linden (143) administered to rabbits six subcutaneous injections of increasing amounts of live or heat-killed organisms of *Monilia albicans*. The injections were given every eight days. After an unspecified length of time the animals were challenged with an intravenous injection of 2.5 times the lethal dose for the same organism. All rabbits died in less than four days presenting typical symptoms of generalized moniliasis.

The information given in all of the

above mentioned papers is not sufficient to permit their adequate evaluation.

Cavallero (10) used three different schedules to immunize rabbits with living cells of *Mycotorula albicans*.

The first group was pretreated with a single intravenous injection of 2 billion blastospores and challenged 10 to 15 days later intravenously with a massive dose (presumably 2 billion blastospores) of a strain of the fungus said to be more pathogenic than the one used for pretreatment.

The second group was given 3 intravenous injections of 1 2 and 2 billion blastospores, respectively of the same strain that was used for the pretreatment in the first group. The second and third injection was given 4 or 5 and 10 or 11 days, respectively after the first one. These rabbits were challenged with 150 million cells of the more pathogenic strain, which was twice its minimum lethal dose, 30 to 45 days after the last injection.

The third group of rabbits was injected either intravenously or subcutaneously with 500 000 1,000,000 and 10 000 000 cells of the more pathogenic strain and challenged 30 to 45 days after the last injection with 150 million cells of the same strain. The timing of the immunizing injections was the same as in the preceding group.

Except for an insignificant number of rabbits in the first group all animals died at about the same time as the untreated controls.

The author noticed that mycotic lesions in pretreated animals had a more hemorrhagic and exudative character than those of control animals. He

concluded that this may be due to a mechanism similar to the Shwartzman phenomenon, and, possibly due to increased susceptibility to infection in pretreated animals. He speculated that some of the rabbits that survived for a longer period of time may have been slightly protected. The author did not exclude the possibility that both increased susceptibility and protection may have been the result of immunization in his experiments.

It is possible that Cavallero (10) may have rendered the rabbits hypersensitive. This is probable especially since he found that their lesions had a more hemorrhagic and exudative character than those of rabbits which were not pretreated. Similar pathologic lesions were observed in sensitized guinea pigs by Vogel and Krehl (145).

Liberation of the endotoxin-like substance from living *Candida* cells may also have been taking place. This substance may have stimulated the growth of *Candida* and its effect may have decreased the protective action of the specific cell wall antigen. It is also possible that the number of cells in the vaccine injections was insufficient.

Hoffmeister Dickgreiser and Gotting (48) applied a combined schedule using first heat killed and later living cells of *Candida albicans*. They immunized 4 rabbits first with heat killed cells, starting with 2 subcutaneous injections of 500 000 cells each on the first and fourth day injecting intravenously 5,000 000 cells on the seventh day and 10 000,000 cells on the eleventh day. In the further course of immunization living cells were used by intravenous route. The animals were

given 10,000,000 cells on the 18th day and 5 billion cells on the 34th day. Six days later they received an intravenous challenge dose containing 5 billion cells. A nonimmunized control rabbit which was given the same challenge dose died 5 days later with symptoms of generalized moniliasis. Two of the four immunized rabbits were killed 11 days after challenge and revealed no signs of moniliasis on autopsy. The fate of the remaining two immunized rabbits was not reported.

In these apparently successful experiments only dead *Candida* cells were used in the first four injections. This eliminated a source of the endotoxin-like substance which would have been constantly liberated from a living antigen capable of multiplication. The living cells contained in successive injections could not, therefore, be stimulated by an uncontrollable supply of the endotoxin-like substance. Quite the opposite may have occurred when the fifth immunizing injection, which contained living cells, was given 7 days after the fourth one, on the 18th day of treatment. The endotoxin-like substance from the preceding injection may have been effective in suppressing the fungicidal growth by the mechanism of nonspecific immunity as demonstrated by own experiments (Figures 2, 3, 4 and 5). By this time specific antibodies may have developed and been instrumental in increasing resistance to the remaining two intravenous injections of living cells.

Wunder (151) immunized rabbits intravenously by either single injection of sublethal number of living *Candida* cells or by repeated intravenous injections of formal-killed cells. The fre-

quency of injections or the number of organisms were not given. After an unspecified length of time the rabbits were challenged with an intravenous injection of 10 to 100 million living cells of *Candida albicans*. Eighteen immunized rabbits, two of which possessed natural agglutinins, and 18 nonimmunized controls died in from 1 to 6 days. It was not stated how many rabbits were immunized with living and how many with dead cells. One immunized rabbit survived. Immunized animals had slightly to moderately elevated agglutinins. The lesions produced in immunized rabbits resembled those noted in the nonimmunized group. The author concluded that agglutinins did not protect the animals from the lethal effects of the organism. He believed that *Candida* elaborated a toxin which was unaffected by agglutinins.

It is impossible to determine the reasons for the poor results of immunization because of insufficient information.

#### (b) Active Immunization with Living Cells in Guinea Pigs

Concetti (14) was unable to protect guinea pigs against challenge by injections of increasing doses of cultures of *O. dem. albicans* attenuated by slow desiccation for 1 or 2 weeks. The injections were given over a period of several weeks every 1 or 2 days subcutaneously or intraperitoneally. Too few data were presented by the author to permit any evaluation of the experiment.

Nirai and Fittipaldi (96) used for their experiments a pathogenic *Candida* which they called *Candida Nirai*.

Fittipaldi (page 28) They noted that two intraperitoneal injections of 1 000 000 000 cells each given 10 days apart kill all animals 1 to 3 days after the second injection. When reinjected in 30 instead of 10 days about 2/3 of the animals died.

In another experiment guinea pigs were injected either with one intraperitoneal injection containing 500 000 000 cells of *Candida* or with four intraperitoneal doses of 50 000 000 cells each and challenged ten or thirty days after the last injection with 100 000 000 cells administered intraperitoneally. The animals were killed 1/2 hour or 3 hours after challenge. Fungi could be found anywhere scattered in the peritoneal cavity in controls which received the challenge dose only while pretreated animals had organisms only in the epiploon. Hemorrhagic exudate was present in the abdominal cavity of pretreated animals while there was none in controls receiving the challenge dose only.

Since the guinea pigs in these experiments of Ninni and Fittipaldi receiving 1 000 000 000 cells did not die within 30 days following the first injection it appears that the infection may have been subsiding. The number of living cells was probably not sufficient to liberate enough endotoxin like substance in the susceptible tissues of these guinea pigs at the time of the second injection to enhance the infection sufficiently as to produce death of all animals. Hyper-sensitivity, an insufficient level of specific antibodies, as well as some effect of the endotoxin-like substance on the depression of non-specific resistance may

have been responsible for the deaths of two thirds of animals which did occur in this group.

In the experiment in which the interval between the two injections was ten days, more endotoxin-like substance may have been present at the time of the second injection. Another factor playing a role in the death of all animals reinjected after 10 days may have been the possibility that this interval was too short for the development of specific immunity.

#### (c) Active Immunization with Living Cells in Mice

Fischer and Horbach (31) have injected 60 mice intraperitoneally with 0.2 cc. of a 4 % suspension of *Candida albicans* (which in own experience contains about 100 000 000 cells). On the 23rd day 33 surviving mice were challenged intraperitoneally with an equal dose of *Candida*. Three weeks later 28 mice were dead while only 19 of 33 control mice died in the same period of time. The authors concluded that specific infection immunity to *Candida* did not exist in mice.

The endotoxin like substance contained in the first dose may have enhanced the growth of organisms in the second dose and caused higher death rate. The role of allergic sensitization in the enhancement of the *Candida* infection may also be considered.

Mourad and Friedman (89) treated mice with 6 subcutaneous injections of vaccines given 40 30 20 12 5 and 1 day before intravenous challenge with  $10^4$  or  $10^5$  viable cells of *Candida albicans*. Three different vaccines were

used viable cells, merthiolate-killed cells and cells vibrated for nearly 7 hours in a sonic oscillator. A single dose of each vaccine corresponded to 40,000,000 cells. Thirty to forty mice were used in each group. All 49 nonvaccinated controls challenged with 100,000 cells died in less than 35 days. One hundred and forty days after challenge the mortality rates were 30% 73% and 77% respectively for mice treated with sonically vibrated, merthiolate-killed and living vaccines. A total of 62% of 45 nonimmunized controls which received an intravenous challenge of 10,000 cells were dead 140 days after they had been infected. The mortality rates of immunized mice in this experiment were 17% 36% and 43% respectively for animals pre-treated with sonically vibrated, merthiolate-killed and living cells. All mice, treated and untreated, had lesions caused by *Candida* indistinguishable from each other.

Among the three groups better results were obtained with sonically vibrated and merthiolate-killed cells than with the viable vaccine. This may have been caused by the fact that the endotoxin-like substance contained in each subsequent injection could not have enhanced the growth of cells in the preceding injections, since they were dead. It is possible that some of the endotoxin-like substance of *Candida* may have been released by sonic vibrations and may have been more easily available from this preparation than from intact live or merthiolate-killed cells. Although the vaccine was injected subcutaneously by route in which endotoxins apparently influence nonspecific resistance to a re-

latively slight extent (62 and Figure 10) it is possible that the endotoxin-like substance may have increased non-specific resistance to the intravenous challenge especially since an injection was given also 5 days before challenge which is usually the time of about the maximum nonspecific enhancement of resistance.

Undoubtedly specific immunity played an important role here and the question is tempting whether a more pronounced protection may not have been obtained by the treatment of the cells with the vibrator by which some of them may have been ruptured and the specific antigen may have become more easily available. An easier availability could be explained on the assumption that the protective antigen may be located in an inside layer of the cell walls which may have become exposed to the tissues of the host after mechanical disintegration of the cells.

A rather success of the immunization with living cells which, as all other vaccines of Mourad and Friedman, were injected subcutaneously as compared to the experiments of others who have utilized the intraperitoneal or intravenous route may be explained by the fact that living cells injected into the subcutaneous space could not easily spread and cause generalized moniliasis. Moreover as pointed out before, the endotoxin is only slightly effective in its action on nonspecific resistance when given subcutaneously. Furthermore the endotoxin-like substance is obviously less easily available from viable intact cells than from a vaccine prepared by sonic vibration.



Fittipaldi (page 28) They noted that two intraperitoneal injections of 1 000 000 000 cells each given 10 days apart kill all animals 1 to 3 days after the second injection. When reinjected in 30 instead of 10 days about 2/3 of the animals died.

In another experiment guinea pigs were injected either with one intraperitoneal injection containing 500 000 000 cells of *Candida* or with four intraperitoneal doses of 50 000 000 cells each and challenged ten or thirty days after the last injection with 100 000 000 cells administered intraperitoneally. The animals were killed 1/2 hour or 3 hours after challenge. Fungi could be found anywhere scattered in the peritoneal cavity in controls which received the challenge dose only while pretreated animals had organisms only in the epiploon. Hemorrhagic exudate was present in the abdominal cavity of pretreated animals while there was none in controls receiving the challenge dose only.

Since the guinea pigs in these experiments of Niani and Fittipaldi receiving 1 000 000 000 cells did not die within 30 days following the first injection it appears that the infection may have been subsiding. The number of living cells was probably not sufficient to liberate enough endotoxin like substance in the susceptible tissues of these guinea pigs at the time of the second injection to enhance the infection sufficiently as to produce death of all animals. Hyper sensitivity an insufficient level of specific antibodies, as well as some effect of the endotoxin-like substance on the depression of nonspecific resistance may

have been responsible for the deaths of two thirds of animals which did occur in this group.

In the experiment in which the interval between the two injections was ten days, more endotoxin-like substance may have been present at the time of the second injection. Another factor playing a role in the death of all animals re-injected after 10 days may have been the possibility that this interval was too short for the development of specific immunity.

#### (c) Active Immunization with Living Cells in Mice

Fischer and Horbach (31) have injected 60 mice intraperitoneally with 0.2 cc. of a 4 % suspension of *Candida albicans* (which in own experience contains about 100 000 000 cells). On the 23rd day 33 surviving mice were challenged intraperitoneally with an equal dose of *Candida*. Three weeks later 28 mice were dead while only 19 of 33 control mice died in the same period of time. The authors concluded that specific infection immunity to *Candida* did not exist in mice.

The endotoxin-like substance contained in the first dose may have enhanced the growth of organisms in the second dose and caused higher death rate. The role of allergic sensitization in the enhancement of the *Candida* infection may also be considered.

Mourad and Friedman (89) treated mice with 6 subcutaneous injections of vaccines given 40 30 20 12 5 and 1 day before intravenous challenge with  $10^4$  or  $10^5$  viable cells of *Candida albicans*. Three different vaccines were

used viable cells, merthiolate-killed cells and cells vibrated for nearly 7 hours in a sonic oscillator. A single dose of each vaccine corresponded to 40,000,000 cells. Thirty to forty mice were used in each group. All 49 nonvaccinated controls challenged with 100,000 cells died in less than 55 days. One hundred and forty days after challenge the mortality rates were 50%, 73% and 77% respectively for mice treated with sonically vibrated, merthiolate-killed and living vaccines. A total of 62% of 43 nonimmunized controls which received an intravenous challenge of 10,000 cells were dead 140 days after they had been infected. The mortality rates of immunized mice in this experiment were 17%, 36% and 43% respectively for animals pre-treated with sonically vibrated, merthiolate-killed and living cells. All mice, treated and untreated, had lesions caused by *Candida* indistinguishable from each other.

Among the three groups better results were obtained with sonically vibrated and merthiolate-killed cells than with the viable vaccine. This may have been caused by the fact that the endotoxin-like substance contained in each subsequent injection could not have enhanced the growth of cells in the preceding injections, since they were dead. It is possible that some of the endotoxin-like substance of *Candida* may have been released by sonic vibrations and may have been more easily available from this preparation than from intact live or merthiolate-killed cells. Although the vaccine was injected subcutaneously by the route in which endotoxins apparently influence nonspecific resistance to a re-

latively slight extent (62 and Figure 10) it is possible that the endotoxin-like substance may have increased nonspecific resistance to the intravenous challenge, especially since an injection was given also 5 days before challenge which is usually the time of about the maximum nonspecific enhancement of resistance.

Undoubtedly specific immunity played an important role here and the question is tempting whether a more pronounced protection may not have been obtained by the treatment of the cells with the vibrator by which some of them may have been ruptured and the specific antigen may have become more easily available. An easier availability could be explained on the assumption that the protective antigen may be located in an inside layer of the cell walls which may have become exposed to the tissues of the host after mechanical disintegration of the cells.

A relative success of the immunization with living cells which, as all other vaccines of Mourad and Friedman, were injected subcutaneously as compared to the experiments of others who have utilized the intraperitoneal or intravenous route may be explained by the fact that living cells injected into the subcutaneous space could not easily spread and cause generalized moniliasis. Moreover as pointed out before, the endotoxin is only slightly effective in its action on nonspecific resistance when given subcutaneously. Furthermore, the endotoxin-like substance is obviously less easily available from viable intact cells than from a vaccine prepared by sonic vibration.

In the case of the merthiolate treated vaccine with which the results were slightly better than with the live vaccine there were no living cells in the immunizing injections to be enhanced by the nonspecific mechanism.

#### (d) Active Immunization with Dead Cells in Rabbits

Some reports are also available on active immunization of animals with dead cells. Ostrovsky (99) reported that a rabbit repeatedly inoculated by the subcutaneous route over a period of one and a half months with 50 cc. of heat killed cells did not survive a challenge of 2 cc. of a virulent culture. His serum was not fungistatic. The number of cells, number and frequency of injections or the interval between the last injection and the challenge was not given. Another rabbit similarly treated did not survive the challenge.

Connor (15) immunized a rabbit with 41 cc. of a suspension of heat killed cells of *Afonilia psilans* divided into 6 doses over a period of 6 weeks. The number of organisms contained in this vaccine was not given. The animal was challenged after an unspecified length of time with an intravenous injection of living cells and died in 4 days, the same length of time as a nonimmunized control. His lesions were less extensive than those of the control rabbit. The author concluded that this was an expression of a slight relative immunity.

The unsuccessful immunization experiments of van der Linden (143) in which the author used also heat killed *Afonilia albicans* besides a viable vaccine have been mentioned in the section deal-

ing with immunization with viable cells (page 35). The injection schedules and the results were the same for live and dead cells.

The experiments of the three preceding authors cannot be adequately evaluated because of lack of specific data.

Cavallero (10) whose experiments were also mentioned in the preceding section (page 36) used heat killed cells besides live ones for immunization. He injected rabbits intravenously with 25, 50 and 150 million heat killed cells of a strain of *Mycotorula albicans* which had a high pathogenicity. The second and third injections were given 4 or 5 and 10 or 11 days, respectively after the first one. The animals were challenged with 150 million living cells of the homologous strain which was twice its minimum lethal dose 30 to 45 days after the last vaccine injection. All animals died at about the same time as the untreated controls. There was no difference in the results between rabbits immunized with living or heat killed cells.

Because of the long interval between the last immunizing injection and challenge (30 to 45 days) nonspecific immunity was apparently not playing any role in these experiments. The factors responsible for the negative results may have been allergic sensitization and, possibly, insufficient numbers of cells contained in the vaccine injections.

Hurd and Drake (53) immunized actively 2 rabbits by treating them intravenously for almost 9 months with an unspecified number of injections of heat killed cells of *Candida albicans* preserved with formalin. Six days after the last injection they received a chal-

large dose of *C. albicans*. Both died in less than 48 hours. The authors concluded that active immunization was ineffective and possibly deleterious.

The immunization experiments of Winner (151) with a single intravenous injection of living organisms have been mentioned on page 57. The author used also repeated intravenous injections of formal-killed cells of *Candida albicans* and concluded that immunization did not protect rabbits against fatal challenge.

Neither the experiments of Hurd and Drake nor those of Winner may be evaluated because of lack of data. It is possible that the rabbits of Hurd and Drake may have become hypersensitive.

#### ( ) Active Immunization with Dead Cells in Guinea Pigs

Mazzetti and Fina Mazzettini (83) have immunized guinea pigs by injecting them subcutaneously with 250 and 500 million or 250, 500 and 1,000 million formalin-killed cells of *Candida albicans* or *C. paratuberculosis* given at intervals of 7 days. Twelve days after the last injection all animals were given an intravenous challenge of living culture of *C. albicans* which killed controls between 7 and 14 days. The number of animals used or the size of challenge dose was not stated except that it was a 1:250 dilution of 24 hour agar culture. At the time of death of the last control animal all immunized guinea pigs were alive. On autopsy all organs were negative for fungi on culture except for one animal immunized with two doses of *C. paratuberculosis*.

These experiments may have been

successful because no living cells were used which may have been enhanced by the endotoxin-like substance contained in the preceding injections. If the conclusions of Kurotchkin and Lim (67) are correct, the guinea pigs in this experiment may not have been strongly sensitized since formalin was used to kill the cells. The number of cells used for immunization was higher and therefore apparently more adequate than in most other experiments. It is possible that since the challenge dose was given 12 days after the last immunizing injection the nonspecific effect of the endotoxin may still have been operative in increasing resistance.

#### (f) Active Immunization with Dead Cells in Mice

Marcus and Rambo (80) injected mice intraperitoneally once weekly for 3 weeks with formalin-killed suspensions of yeast cells of *Candida albicans*. The mice were challenged after an unspecified length of time intravenously with one or more LD<sub>50</sub> of the living organism. There was no significant difference between the mortality rates of immunized and control animals but kidney cultures of immunized mice were usually negative unlike those of control animals.

Hill (46) immunized mice with formalin-killed cells of *Candida albicans* injected intraperitoneally and found slightly lower mortality rates than in controls. Organs of immunized animals contained fewer viable organisms than those of nonimmunized controls.

Because of insufficient information a critical appraisal of these experiments is impossible.

In the case of the merthiolate-treated vaccine with which the results were slightly better than with the live vaccine there were no living cells in the immunizing injections to be enhanced by the nonspecific mechanism.

#### (d) Active Immunization with Dead Cells in Rabbits

Some reports are also available on active immunization of animals with dead cells. Ostrovsky (99) reported that a rabbit repeatedly inoculated by the subcutaneous route over a period of one and a half months with 50 cc. of heat killed cells did not survive a challenge of 2 cc. of a virulent culture. His serum was not fungistatic. The number of cells, number and frequency of injections or the interval between the last injection and the challenge was not given. Another rabbit similarly treated did not survive the challenge.

Connor (15) immunized a rabbit with 41 cc. of a suspension of heat killed cells of *Monilia psalms* divided into 6 doses over a period of 6 weeks. The number of organisms contained in this vaccine was not given. The animal was challenged after an unspecified length of time with an intravenous injection of living cells and died in 4 days, the same length of time as a nonimmunized control. His lesions were less extensive than those of the control rabbit. The author concluded that this was an expression of a slight relative immunity.

The unsuccessful immunization experiments of van der Linden (143) in which the author used also heat killed *Monilia albicans* besides a viable vaccine have been mentioned in the section deal-

ing with immunization with viable cells (page 35). The injection schedules and the results were the same for live and dead cells.

The experiments of the three preceding authors cannot be adequately evaluated because of lack of specific data.

Cavallero (10) whose experiments were also mentioned in the preceding section (page 36) used heat killed cells besides live ones for immunization. He injected rabbits intravenously with 25, 50 and 150 million heat killed cells of a strain of *Myxotolula albicans* which had a high pathogenicity. The second and third injections were given 4 or 5 and 10 or 11 days, respectively, after the first one. The animals were challenged with 150 million living cells of the homologous strain which was twice its minimum lethal dose, 30 to 45 days after the last vaccine injection. All animals died at about the same time as the untreated controls. There was no difference in the results between rabbits immunized with living or heat-killed cells.

Because of the long interval between the last immunizing injection and challenge (30 to 45 days) nonspecific immunity was apparently not playing any role in these experiments. The factors responsible for the negative results may have been allergic sensitization and, possibly, insufficient numbers of cells contained in the vaccine injections.

Hurd and Drake (53) immunized actively 2 rabbits by treating them intravenously for almost 9 months with an unspecified number of injection of heat killed cells of *Candida albicans* preserved with formalin. Six days after the last injection they received a chal-

large dose of *C. albicans*. Both died in less than 48 hours. The authors concluded that active immunization was ineffective and possibly deleterious.

The immunization experiments of Winner (151) with single intravenous injection of living organisms have been mentioned on page 37. The author used also repeated intravenous injections of formalin-killed cells of *Candida albicans* and concluded that immunization did not protect rabbits against fatal challenge.

Neither the experiments of Hurd and Drake nor those of Winner may be evaluated because of lack of data. It is possible that the rabbits of Hurd and Drake may have become hyperimmune.

#### (c) Active Immunization with Dead Cells: Guinea Pigs

Mazzetti and Fissi Mazzaccini (83) have immunized guinea pigs by injecting them subcutaneously with 250 and 500 million or 250, 500 and 1,000 million formalin-killed cells of *Candida albicans* or *C. parapsilosis* given at intervals of 7 days. Two days after the last injection all animals were given an intravenous challenge of a living culture of *C. albicans* which killed controls between 7 and 14 days. The number of animals used at the time of challenge dose was not stated except that it was a 1:250 dilution of 24 hour agar culture. At the time of death of the last control animal all immunized guinea pigs were alive. On autopsy all organs were negative for fungi on culture except for one animal immunized with two doses of *C. parapsilosis*.

These experiments may have been

successful because no living cells were used which may have been enhanced by the endotoxin-like substance contained in the preceding injections. If the conclusions of Kurotchkin and Lim (67) are correct, the guinea pigs in this experiment may not have been strongly sensitized since formalin was used to kill the cells. The number of cells used for immunization was higher and therefore apparently more adequate than in most other experiments. It is possible that since the challenge dose was given 12 days after the last immunizing injection the nonspecific effect of the endotoxin may still have been operative in increasing resistance.

#### (i) Active Immunization with Dead Cells: Mice

Marcus and Rambo (80) injected mice intraperitoneally once weekly for 3 weeks with formalin-killed suspensions of yeast cells of *Candida albicans*. The mice were challenged after an unspecified length of time intravenously with one or more LD<sub>50</sub> of the living organism. There was no significant difference between the mortality rates of immunized and control animals but kidney cultures of immunized mice were usually negative unlike those of control animals.

Hill (46) immunized mice with formalin-killed cells of *Candida albicans* injected intraperitoneally and found slightly lower mortality rates than in controls. Organs of immunized animals contained fewer viable organisms than those of nonimmunized controls.

Because of insufficient information a critical appraisal of these experiments is impossible.

The experiments of Mourad and Friedman (89) have been discussed in the section on immunization with living cells (pages 38—40). As was pointed out there, mortality rates of mice immunized with merthiolate-killed and sonically vibrated cells were lower than of those immunized with living cells.

(g) *Active Immunization with Fractions or Products of Candida Cells*

Attempts have been made to produce active specific immunity in animals by the use of fractions of cells of *Candida* or their cellular products.

Ostrovsky (99) reported that a single subcutaneous injection of 20 cc. of a culture filtrate of *Oidium albicans* did not immunize a rabbit.

Concetti (14) attempted, also unsuccessfully, to immunize guinea pigs with repeated subcutaneous injections of culture filtrates of *Oidium albicans* against the same fungus injected intraperitoneally.

The same author injected subcutaneously into rabbits increasing doses of a saline suspension of a sediment remaining after grinding of dried *Oidium* cells in a mortar. The highest dose of this suspension was 2 cc. the weight of solids contained in this volume was not given. After an unspecified length of time rabbits were challenged intravenously with 2 cc. of a viable *Oidium* culture an amount which killed controls in 2 to 5 days. All six treated rabbits survived and manifested only a slight loss of weight.

These successful experiments are in agreement with own experimental results in which suspensions of lyophilized

washed cell walls were used for active immunization of mice (Figure 1).

(h) *Active Immunization of Animals Already Infected with Candida*

Attempts have also been made to induce active immunity against *Candida* by vaccine treatment in animals after they had been infected with the fungus.

Concetti (14) infected two rabbits by intravenous injections of 2 cc. of a viable culture of *Oidium albicans*. One day later he started treating these animals with increasing doses of a saline suspension of a sediment remaining after grinding of dried *Oidium* cells. Daily injections were given for 4 days, starting with 1/4 cc. the highest amount being 1 cc. While two control animals died on the second and third day respectively one treated rabbit died on the 7th, the other on the 8th day. Lesions caused by *Oidium* were less pronounced in treated animals than in controls.

Fomina and Stepanishcheva (34) infected mice with 1.5 million living cells of *Candida albicans*. Two hours later the authors started treating the animals by active immunization with a suspension of heat killed *Candida* cells which was administered intravenously and subcutaneously every 1 to 3 days for 1 1/2 months. The doses ranged from 200,000 to 8,000,000 cells. One and a half months after the experiment was started 28% of the vaccine-treated mice survived while there were only 8% survivors in the control group.

The experiments of Concetti and Fomina and Stepanishcheva suggest that vaccine therapy of moniliasis may be feasible.

### (ii) Passive Immunization

Passive immunization was attempted in rabbits and mice. del Pont (16) treated rabbits with cultures of *Endomycis albicans* which had been attenuated by heating at 35 C for 10 minutes. The minimal lethal dose of the untreated culture of this strain of the fungus was 0.2 cc. The author injected increasing amounts of the heated suspension of *Endomycis* every 6 to 7 days for an unspecified length of time, starting with 0.2 cc. until he reached 5 cc.

The serum of animals thus treated was used for intravenous injections on four rabbits. They received 1, 2, 5 and 10 cc. of the immune serum, respectively. Twenty-four hours later they were challenged intravenously together with one control animal which did not receive any serum, with 0.5 cc. of a virulent culture of the same fungus. The untreated control rabbit died five days later. The animal which received 5 cc. of the serum died 4 days after challenge with typical symptoms of mycosis. The rabbit which was passively immunized with 1 cc. of immune serum died 25 days after challenge, apparently of an unrelated bacterial infection. The other two rabbits remained in good health for the duration of the experiment which was 4 months.

These experiments suggest that most of the animals may have been protected against lethal challenge by pretreatment with passively transferred antibodies. The rabbit which received 10 cc. of serum and died may have become sufficiently hypersensitive by passive transfer and susceptible to challenge.

Hoffmeister, Dickmeyer and Götting

(18) used the serum of two of the four rabbits which were actively immunized by a combined schedule of injections of heat-killed and living cells of *Candida albicans* which was described in a previous section (pages 36-37). The authors injected 20 cc. of this immune serum intravenously to each of two rabbits together with 5 billion and 7.5 billion of living cells, respectively. None of the passively immunized animals showed any signs of the disease after 30 days while one control rabbit died in 4 and another in 7 days.

In these experiments the immune serum and challenge were both given simultaneously by intravenous route. It is possible that the antiserum agglutinated the living cells and reduced thus the size of the inoculum.

Hurd and Drake (53) used serum withdrawn from 2 rabbits which were actively immunized over a period of almost 9 months by repeated injections of a vaccine prepared from *Candida albicans* treated by heat and formalin (page 40). The serum of these two rabbits which had an agglutinin titer of 1:1,80 and 1:2,560, respectively was injected into other rabbits. These were infected immediately after the serum injection with an amount of living cells of *C. albicans* which killed 8 of 10 controls within a week. Three of five rabbits which received 1 ml. of antiserum and *Candida* died in 5 to 6 days. The two remaining animals were killed after an unspecified length of time. Four of five rabbits which were given 3 ml. of antiserum and *Candida* also died in 5 to 6 days. The surviving rabbit was killed after ten days. The three



The experiments of Mourad and Friedman (89) have been discussed in the section on immunization with living cells (pages 38—40). As was pointed out there, mortality rates of mice immunized with merthiolate killed and sonically vibrated cells were lower than of those immunized with living cells.

(g) *Active Immunization with Fractions or Products of Candida Cells*

Attempts have been made to produce active specific immunity in animals by the use of fractions of cells of *Candida* or their cellular products.

Ostrovsky (99) reported that a single subcutaneous injection of 20 cc. of a culture filtrate of *Oidium albicans* did not immunize a rabbit.

Concetti (14) attempted, also unsuccessfully to immunize guinea pigs with repeated subcutaneous injections of culture filtrates of *Oidium albicans* against the same fungus injected intraperitoneally.

The same author injected subcutaneously into rabbits increasing doses of a saline suspension of a sediment remaining after grinding of dried *Oidium* cells in a mortar. The highest dose of this suspension was 2 cc. the weight of solids contained in this volume was not given. After an unspecified length of time rabbits were challenged intravenously with 2 cc. of a viable *Oidium* culture, an amount which killed controls in 2 to 5 days. All six treated rabbits survived and manifested only a slight loss of weight.

These successful experiments are in agreement with own experimental results in which suspensions of lyophilized

washed cell walls were used for active immunization of mice (Figure 1)

(h) *Active Immunization of Animals Already Infected with Candida*

Attempts have also been made to induce active immunity against *Candida* by vaccine treatment in animals after they had been infected with the fungus.

Concetti (14) infected two rabbits by intravenous injections of 2 cc. of a viable culture of *Oidium albicans*. One day later he started treating these animals with increasing doses of a saline suspension of a sediment remaining after grinding of dried *Oidium* cells. Daily injections were given for 4 days, starting with 1/4 cc. the highest amount being 1 cc. While two control animals died on the second and third day respectively, one treated rabbit died on the 7th, the other on the 8th day. Lesions caused by *Oidium* were less pronounced in treated animals than in controls.

Fomina and Stepanushcheva (34) infected mice with 1.5 million living cells of *Candida albicans*. Two hours later the authors started treating the animals by active immunization with a suspension of heat killed *Candida* cells which was administered intravenously and subcutaneously every 1 to 3 days for 1 1/2 months. The doses ranged from 200 000 to 8 000 000 cells. One and a half months after the experiment was started 28 % of the vaccine-treated mice survived while there were only 8 % survivors in the control group.

The experiments of Concetti and Fomina and Stepanushcheva suggest that vaccine therapy of moniliasis may be feasible.

cells of *Monilia pilous*. After a length of time which was not given two of these mice received also 0.2 cc. and 0.4 cc., respectively of serum from a patient who had a chronic osteomyelitis due to *M. pilous*. The two which received no serum and the mouse which was given 0.2 cc. of the serum revealed lesions due to the fungus on autopsy which was performed two weeks later. The fourth mouse which had received 0.4 cc. of the serum contained no lesions.

Fomina and Surpanishcheva (34) treated a large series of mice with anti-*Candida albicans* rabbit serum diluted 1:10 with saline in daily doses increasing from 0.1 to 1 cc. These mice were injected intravenously with 25 million living cells of *C. albicans* 2 hours before the first serum injection was given. The source of the serum were rabbits which had undergone a long-term immunization with killed or living cells of *Candida*. On the twentieth day of the experiment 20–25% of the mice were still alive but all of them died between the 30th and 40th day. All untreated control mice died between the 5th and the 9th day of the experiment.

The limited experiments of Connor (15) and those of Fomina and Surpanishcheva (34) point to the possibility that serum therapy of previously infected animals may be feasible.

#### (D) Immunization in Humans

No reports could be located in the literature on the prevention of active or passive immunization in human moniliasis. Reports on the therapeutic use of vaccines prepared from *Candida* are not

numerous. Their mode of action, at least in some instances, may be interpreted as desensitization rather than active specific immunization.

#### (1) Active Immunization

Vaccine therapy was reported by Gross and Balog (39) who treated twelve patients suffering from pulmonary moniliasis, with increasing doses of living yeast cells of *Monilia albicans* intracutaneously and subcutaneously. The suspension contained a loopful of culture material in 3 cc. of saline solution. Injections were started with 0.1 cc., and the dose was gradually increased to 1 cc. The authors claimed improvement or cure of all treated patients. Some of them, however apparently also received iodides.

Emdin and Finlayson (25) treated a 4-year-old boy with an ulcerative skin lesion on the upper lip and meningitis, both due to *Candida albicans*. Local measures and systemic treatment were applied. The latter consisted of penicillin, potassium iodide, intravenous gentian violet and a *Candida* vaccine. The vaccine was prepared from heat killed cells as described by Connor et al. (15). A dilution of 1:1,000 of a standardized stock vaccine (1:1,000) was used at first, commencing with 0.1 ml. and increasing the dose by this amount every second day. A more concentrated vaccine was injected later. Vaccines and iodide therapy were continued for several months. No information is given about the highest concentration of the vaccine or the total number of injections given. When the child was discharged after about 5 months of hospitalization he

surviving animals in these 2 groups had almost or completely healed lesions. Two rabbits were treated with daily intravenous injections of antiserum until death which occurred within a week. The authors concluded that passive immunization was ineffective and possibly deleterious.

They noticed, however some morphologic peculiarities of the lesions in passively immunized animals, viz. thick walled, distorted blastospores, a tendency of the organisms to form packed masses, and necrosis in the centers of some fungal lesions. A similar morphology of the lesions was found in some non-immunized rabbits surviving for longer periods of time, possibly indicating the building up of natural antibodies.

The authors did not give enough information about the method of immunization of rabbits which were used as the source of antiserum. Although they were unable to prolong the life of the majority of passively immunized rabbits 3 of 10 animals survived for longer periods than the controls. The failure to protect most rabbits may have been caused by an insufficient amount of antiserum or a passive transfer of hypersensitivity.

Ata (1) reported that mice pretreated with anti *Candida albicans* rabbit serum and challenged with  $5 \times 10^5$  cells of *C. albicans* had lower mortality rates than controls pretreated with normal serum. Mortality of mice injected with 0.1 cc. of anti-tuberculous rabbit serum intraperitoneally and challenged with an intravenous injection of  $3 \times 10^7$  cells of *C. albicans* 30 minutes later was 40 % fifteen days after the injection while

controls injected with normal rabbit serum had a mortality of 80 %

The protection experiments with anti-*Candida* serum confirm the positive results of del Pont (16). The experimental results according to which mice injected with antituberculous rabbit serum were partly protected against a challenge with *Candida* may possibly be explained by the transfer of non-specific resistance from rabbits pretreated with the Koch bacillus. This appears possible in view of the experimental results of Benacerraf, Sebestyen and Schlossman (3) who found that intravenous injections of serum from endotoxin-treated mice into normal mice transfer the enhanced ability to clear *Escherichia coli* from the blood. It will be mentioned later (page 51) that *Mycobacterium tuberculosis* or its extracts may modify nonspecific host resistance in a similar manner as the endotoxins of gram-negative bacteria (20).

Hasenclever and Mitchell (40) injected 29 mice intraperitoneally with 0.5 cc. of anti *Candida albicans* rabbit serum diluted 1:5 two hours before an intravenous challenge with  $10^7$  cells of *C. albicans*. This pretreatment did not protect them from the lethal effects of the challenge. It is possible that passive transfer of hypersensitivity to mice may have taken place.

The experiments of the following two authors deal with serum therapy of animals previously infected with *Candida*.

Connor (15) injected intraperitoneally 4 mice with 0.2 cc. of a suspension containing an unspecified number of viable

*albicans* killed with formal and heat. The tested persons received 5 doses containing 500 million, 1 2, 3 and 4 billion cells, respectively in 1 cc. The injections were given every 3 days. The purpose of the study was to examine the immunological response of humans to the vaccine. It was found that with the exception of one subject an elevation of agglutinating and complement fixing titers took place in all tested persons after the treatment. The majority of them developed local erythema and infiltration at the site of the injections. General reactions were slight, consisting occasionally of moderate hyperthermia and slight headache. The previously negative skin tests became positive only in some cases, and the elevation of gamma globulin was inconsistent.

It appears that while the number of cells injected to the patients of Gromi and Balog (39) and Emdin and Finlayson (25) was too small to produce specific immunity and the effect of the therapy may be probably attributed to desensitization, the dosage in the vaccine treatment of Scuro, Perrone and Zam-

boni (130) may have been high enough to produce specific immunization.

## (ii) Passive Immunization

Hlatt and Martin (45) treated an adult patient with pulmonary moniliasis by the subcutaneous administration of anti-*Candida albicans* rabbit serum, beginning with 0.1 cc. of a 1:10 dilution. The dose was increased by 0.1 cc. daily until an amount of 0.9 cc. was reached. This series was repeated twice. The authors claimed a total remission of all symptoms following serum therapy.

Other forms of treatment were also administered prior to the serum therapy viz. iodides, intravenous gentian violet and general supportive measures. These may have accounted for some of the improvement. The amount of serum injected to this patient was very small in comparison with that administered in the animal experiments described on pages 43—45. It is questionable whether a sufficient amount of specific antibodies was transferred passively by this serum therapy.

was in an excellent condition. The authors state that a distinct improvement was noted after a few vaccine injections, so that immunological response may have been the deciding factor in promoting recovery.

The evaluation of the therapeutic effectiveness of the vaccine is difficult since several other forms of therapy were administered. It appears that the total number of cells contained in the vaccine injections was relatively small the initial number being about 1,250 and, in case the maximum amount (1 cc.) of undiluted vaccine was given, which does not seem to be probable, the highest number of dead cells administered would have been about 12,500,000. This is only a small fraction of the number of cells from which cell wall material for specific immunization of mice in own experiments was prepared, and a small fraction of the number of cells used in the few successful immunization experiments in animals [Hoffmeister, Dickgreiser and Gotting (48), Mazzetti and Fissi-Marraccini (83)]. It appears, therefore, that whatever effect the vaccine therapy may have had it may be attributed to desensitization rather than active specific immunization. In view of the work of Kurotchkin and Lim (68) (page 39) such desensitization may be considered helpful.

Although it is difficult to estimate the exact number of cells contained in the vaccines used by Grossi and Balog (39) the same evaluation may apply to their treatments.

Thiers, Coudert, Colomb, Fayolle and Moulin (140) reported good results in treating patients suffering of moniliais

with "clasovaccins" according to Jausson and Boidé (56) which are prepared by hydrolysis of *Candida* with sulfuric or nitric acid which is precipitated by calcium carbonate. The authors state that all allergens are destroyed by this rather brutal method of preparation but that the protective properties of *Candida* are preserved. Unlike with their previous method of vaccine therapy in which they used cultures of *Candida* to which chloroform and formalin were added they did not experience any cutaneous or general allergic reactions, including abdominal pains and diarrhea. The authors maintain that there is a dissociation between the allergenic and protective properties of *Candida*. Details of therapeutic results are not given.

It is possible that the claimed effectiveness of the "clasovaccins" of Jausson against dermatomycoses caused by *Trichophyton tonsurans*, *T. mentagrophytes*, *T. violaceum*, *Epidermophyton floccosum* against sporotrichosis and in cases of secondary eczematoid allergic reactions due to a primary fungal focus (57) as well as the favorable results of a similarly prepared vaccine against moniliais (140) may indeed be based on the absence of sensitizing materials and of endotoxin like substances which may enhance the growth of fungi and on the presence of cell wall material containing the specific protective antigen.

Although Scuro, Perrone and Zamboni (130) used normal subjects for their vaccination the data about side effects in the vaccinated subjects are useful. The authors injected 18 subjects subcutaneously with a suspension of increasing numbers of cells of *Candida*

*albicans* killed with formal and heat. The tested persons received 5 doses containing 500 million, 1 2, 3 and 4 billion cells, respectively in 1 cc. The injections were given every 3 days. The purpose of the study was to examine the immunological response of humans to the vaccine. It was found that with the exception of one subject an elevation of agglutinating and complement fixing titers took place in all tested persons after the treatment. The majority of them developed local erythema and infiltration at the site of the injections. General reactions were slight, consisting occasionally of moderate hyperthermia and slight headache. The previously negative skin tests became positive only in some cases, and the elevation of gamma globulin was inconsistent.

It appears that while the number of cells injected to the patients of Gross and Balog (39) and Erdem and Finlayson (25) was too small to produce specific immunity and the effect of the therapy may be probably attributed to desensitization, the dosage in the vaccine treatment of Scuro, Perrone and Zam-

boni (130) may have been high enough to produce specific immunization.

#### (ii) *Passive Immunization*

Hjatt and Martin (45) treated an adult patient with pulmonary monilliasis by the subcutaneous administration of anti-*Candida albicans* rabbit serum, beginning with 0.1 cc. of a 1:10 dilution. The dose was increased by 0.1 cc. daily until an amount of 0.9 cc. was reached. This series was repeated twice. The authors claimed a total remission of all symptoms following serum therapy.

Other forms of treatment were also administered prior to the serum therapy viz. iodides, intravenous gentian violet and general supportive measures. These may have accounted for some of the improvement. The amount of serum injected to this patient was very small in comparison with that administered in the animal experiments described on pages 43-45. It is questionable whether a sufficient amount of specific antibodies was transferred passively by this serum therapy.

## (4) DISCUSSION

### (a) *Differences between Natural and Experimental Systemic Moniliasis*

Laboratory methods generally used to infect animals with *Candida* do not reflect accurately the processes taking place in human generalized moniliasis. An experimental model in which systemic moniliasis in animals would be produced in a similar manner as in man namely by generalization through invasion of the blood stream from the gastrointestinal tract, has not been designed. Animals fed with *Candida* by mouth cannot be infected regularly. Most investigators have used an experimental model in which animals have been infected with *Candida* either intravenously or intraperitoneally. In this way generalization takes place much more acutely than in natural human infection. It can be expected that even good specific immunity might not prevent the death of some animals in which acute generalized moniliasis was produced by an injection of a massive inoculum directly into the blood stream.

### (b) *Factors Influencing the Outcome of Specific Immunization*

Of the many reviewed immunization experiments only a few those containing adequate data, may be evaluated.

(1) The endotoxin-like substance of *Candida* contained in the vaccine in injections is a factor which may have

either a favorable or an adverse effect on the result of immunization. The resistance-influencing functions of this endotoxin like substance contained in the soluble extract of *Candida* have not been heretofore systematically explored. They are described in the experimental part of this paper (Figures 2, 3 4 5 8 and 9). Own experiments reveal that the effects of the endotoxin-like substance of *Candida* and of the endotoxins of gram negative bacteria on nonspecific resistance are similar. The role of the latter in nonspecific resistance will be briefly reviewed on pages 57—58.

(a) When animals are immunized with living cells of *Candida* the endotoxin-like substance from these viable cells may enhance the growth of the fungus contained in the challenge dose or in each subsequent vaccine dose containing living organisms provided the level of this endotoxin like substance is high in the tissues at the time of such an injection (pages 28—31). In such instances this endotoxin like substance may have an adverse effect on the results of immunization.

This may be true of the experiments in which Cavallero (10) immunized rabbits with living cells (pages 36, 50 and 51). The lack of protection in the experiments of Ninna and Fittipaldi (9b) in which all guinea pigs receiving two large intraperitoneal injections of *Candida* given 10 days apart died while

2/3 of the animals succumbed when the interval was 30 days, may be based on this mechanism (pages 28—29 and 37—38). Higher mortalities after two large intraperitoneal injections of viable cells of *Candida* given 23 days apart as compared to mortalities after one such injection in the experiments of Flächer and Horbach (31) may have been caused, besides sensitization, by this infection-enhancing property of the endotoxin-like substance (pages 38 and 50—51). A relatively lower degree of resistance among mice immunized with living cells, as compared to those immunized with nonviable vaccines, in the experiments of Mourad and Friedman (89) may have been due to the presence of the endotoxin-like substance enhancing the growth of organisms used for immunization and challenge (pages 38—40 49—50 51 52).

(b) In some experiments the endotoxin-like substance may have played a favorable role. When vaccine injections are properly timed, the substance may enhance nonspecific resistance and potentiate the specifically immunizing role of the cell walls. An own experiment has shown that it is not destroyed by heat (Figure 4) and it is, therefore, present in heat-killed vaccines.

In the experiments of Hoffmeister Dickpeter and Göting (48) (pages 36—37 and 51) the first three injections contained dead cells only. The enhancing role of the endotoxin-like substance was thus eliminated. The growth of organisms administered in the fourth injection which contained living cells may have been depressed by the endotoxin-like substance in the third

injection which was given 7 days earlier. This is probable especially since it was administered intravenously by a route by which the resistance-influencing effects of the endotoxin-like substance are relatively pronounced. Specific antibodies may have been instrumental in depressing the growth of organisms in the last two injections. It appears, therefore, that the success of this experiment may have been due to a combination of effects of specific and nonspecific immunity.

Since there was an interval of 12 days between the last vaccine injection and the challenge dose in the experiments of Marretti and Fiesi Maracci (85) (pages 41 and 51) who used dead cells in guinea pigs it is possible that the last vaccine injection may have enhanced resistance to the challenge. The protection achieved in these experiments may thus have also been due to a combination of mechanisms of specific and nonspecific immunity.

Two preparations of dead cells (sonically fibrated and mercuric chloride-killed) were more effective for immunization of mice than viable cells of *Candida* in the experiments of Mourad and Friedman (89) (pages 38—40, 51 and 52). A reason for better results of vaccination with nonviable cells may have been that the endotoxin-like substance in the dead cells could not have enhanced the growth of organisms used for immunization, since it was not constantly produced. The endotoxin-like substance may have even increased nonspecific resistance to the intravenous challenge since an injection of the



vaccine was also given 5 days before challenge. A combination of the effects of specific and nonspecific resistance may have again been responsible for the results.

Of the two nonviable vaccines considerably better results were obtained with the sonically vibrated cells. Easier availability of the specific antigen in the cell wall may have been one reason for it (page 52). It is possible, however, that some of the endotoxin-like substance may have been released by sonic vibrations and may have become more easily and more promptly available from this preparation than from intact merthiolate-killed cells. Although the injections were given subcutaneously the substance may have increased nonspecific resistance to intravenous challenge, especially since an injection was given also 5 days prior to challenge.

(2) *Allergic sensitization* or its absence is another factor capable of influencing the success of immunization against moniliasis. Henrick (44) postulated that the dissemination of lesions in fungus diseases resulted from hypersensitivity. He believed that the marked tendency to give rise to hypersensitivity was the most outstanding characteristic of fungus diseases which distinguished them from most of the other microbial diseases. This points to the relative importance of the elimination of the allergenic fractions of the organisms from antifungal, as compared to antibacterial vaccines, and to the importance of anatomical and chemical identification of the specific antigens in fungi.

It was shown experimentally that hypersensitivity may be one of the factors

that may lead to generalization of *Candida* infections. Kurotchkin and Lim (68) have demonstrated that intratracheal induction of bronchopulmonary moniliasis was possible only after sensitization of rabbits by previous administration of heat killed cells. Ninni and Fittipaldi (96) have shown that, when intraperitoneal injections are properly timed in guinea pigs, immunization with living or heat killed cells may induce marked hemorrhagic lesions in the peritoneum. The experiments of Vogel and Krehl (145) demonstrated hemorrhagic lesions of the lungs following sensitization with heat killed cells. Kesten and Mott (61) were able to sensitize animals actively and passively to *Candida*. According to Kurotchkin and Lim (67) sensitization is less pronounced after treatment with vaccines prepared with formalin. It was shown in the experimental part of this paper that an anaphylactic shock may be produced with the soluble extract of *Candida* but not with the cell walls (Table 1). It may be concluded therefore, that the sensitizing substance from the soluble extract should be excluded from an effective vaccine.

(a) The lack of success of immunization due to sensitization may apply to the experiments of Cavallero (10) (pages 36-48 and 51) who immunized rabbits with either a single intravenous injection of viable cells followed 10-15 days later by a challenge, or with 3 intravenous or subcutaneous injections of viable or heat killed cells given every 4-5 days followed by a challenge 30-45 days later. The same explanation may apply to the experiments of Facher

and Horbach (31). These authors have shown that the number of controls dying after one large intraperitoneal injection of living *Candida* cells is much lower than that of mice dying after the second of two such injections if it follows the first one 23 days later (pages 38 and 49).

(b) At least a partial elimination of allergic sensitization, on the other hand, may have been responsible for the success in some experiments. The use of formalin-killed cells by Mazzetti and Fisi Marracchini (83) (pages 41 and 49) may have decreased the adverse role of sensitization. The treatment of fungal cells with sulfuric acid is the accurate therapy of Thiers, Coudert, Colomb, Fayolle and Moulin (140) (page 46) may have destroyed the sensitizing materials. There is a possibility that sensitization may not have developed because of the gradual increase in dosage and change in route of administration from subcutaneous to intravenous in the experiments of Hoffmeister Dickgiesser and Göting (48) (pages 36—37 and 49).

(3) The *route of administration* of the vaccines is also important.

(a) The fact that the endotoxin-like substance is much more effective in modifying infections when administered intravenously or intraperitoneally than by the subcutaneous route (62 and Figure 10) and that intraperitoneal or intravenous injections of viable organisms may cause generalized moniliasis more easily than subcutaneous ones, may be partly responsible for the lack of success in some immunization experiments.

This may apply to the intraperitoneal injections of living cells in rabbits of Cavallero (10) (pages 36—48 and 50) intraperitoneal injections of living cells in guinea pigs of Nisni and Fitzgaldi (96) (pages 37—38 and 48—49) intraperitoneal injections of living cells in mice of Fischer and Horbach (31) (pages 38, 49 and 50—1).

(b) All injections in successful immunization trials were given subcutaneously except for 4 of 6 injections in the experiments of Hoffmeister Dickgiesser and Göting (48) (pages 36—37 and 49) who started immunizing rabbits subcutaneously and continued by intravenous route.

(4) *Dead cells or their f actions* were used either throughout the immunization or at least during its early stage in all successful experiments. The only exception is one of the experiments of Mourad and Friedman (89) (pages 38—40, 49 and 52) in which slight protection was achieved by subcutaneous injections of viable cells of *Candida*. When dead cells are used a constant production and liberation of the endotoxin-like substance by the cells contained in the vaccine is not possible and its depressing action on nonspecific resistance is thus eliminated.

(5) Another factor necessary for a favorable outcome of immunization is a *sufficient quantity and relative ease of availability of the specific protective antigen*.

The limited experiments of Concetti (14) (page 42) and own findings (Figure 1) demonstrate that cell walls alone may protect against challenge and that other parts of the cell are ap-

vaccine was also given 5 days before challenge. A combination of the effects of specific and nonspecific resistance may have again been responsible for the results.

Of the two nonviable vaccines considerably better results were obtained with the sonically vibrated cells. Easier availability of the specific antigen in the cell wall may have been one reason for it (page 52). It is possible, however, that some of the endotoxin-like substance may have been released by sonic vibrations and may have become more easily and more promptly available from this preparation than from intact merthiolate-killed cells. Although the injections were given subcutaneously the substance may have increased nonspecific resistance to intravenous challenge, especially since an injection was given also 5 days prior to challenge.

(2) *Allergic sensitization* or its absence is another factor capable of influencing the success of immunization against moniliasis. Henrica (44) postulated that the dissemination of lesions in fungus diseases resulted from hypersensitivity. He believed that the marked tendency to give rise to hypersensitivity was the most outstanding characteristic of fungus diseases which distinguished them from most of the other microbial diseases. This points to the relative importance of the elimination of the allergenic fractions of the organisms from antifungal, as compared to antibacterial vaccines, and to the importance of anatomical and chemical identification of the specific antigens in fungi.

It was shown experimentally that hypersensitivity may be one of the factors

that may lead to generalization of *Candida* infections. Kurotchkin and Lim (68) have demonstrated that intratracheal induction of bronchopulmonary moniliasis was possible only after sensitization of rabbits by previous administration of heat killed cells. Ninni and Fittipaldi (96) have shown that, when intraperitoneal injections are properly timed in guinea pigs, immunization with living or heat killed cells may induce marked hemorrhagic lesions in the peritoneum. The experiments of Vogel and Krehl (145) demonstrated hemorrhagic lesions of the lungs following sensitization with heat killed cells. Kesten and Mott (61) were able to sensitize animals actively and passively to *Candida*. According to Kurotchkin and Lim (67) sensitization is less pronounced after treatment with vaccines prepared with formalin. It was shown in the experimental part of this paper that an anaphylactic shock may be produced with the soluble extract of *Candida* but not with the cell walls (Table 1). It may be concluded therefore, that the sensitizing substance from the soluble extract should be excluded from an effective vaccine.

(a) The lack of success of immunization due to sensitization may apply to the experiments of Cavallero (10) (pages 36-48 and 51) who immunized rabbits with either a single intravenous injection of viable cells followed 10-15 days later by a challenge, or with 3 intravenous or subcutaneous injections of viable or heat killed cells given every 4-5 days, followed by a challenge 30-45 days later. The same explanation may apply to the experiments of Fischer

and Horthach (31). These authors have shown that the number of controls dying after one large intraperitoneal injection of living *Candida* cells is much lower than that of mice dying after the second of two such injections if it follows the first one 23 days later (pages 38 and 49).

(b) At least a partial elimination of allergic sensitization, on the other hand, may have been responsible for the success in some experiments. The use of formalin-killed cells by Mazzetti and Fasi Marraccini (83) (pages 41 and 49) may have decreased the adverse role of sensitization. The treatment of fungal cells with sulfuric acid in the adjuvant therapy of Thiers, Coudert, Colomb, Fayolle and Moulin (140) (page 46) may have destroyed the sensitizing materials. There is a possibility that sensitization may not have developed because of the gradual increase in dosage and change in route of administration from subcutaneous to intravenous in the experiments of Hoffmeister, Dickgesser and Göting (48) (pages 36—37 and 49).

(3) The *route of administration* of the vaccine is also important.

(a) The fact that the endotoxin-like substance is much more effective in modifying infections when administered intravenously or intraperitoneally than by the subcutaneous route (62 and Figure 10) and that intraperitoneal or intravenous injections of viable organisms may cause generalized monilliasis more easily than subcutaneous ones, may be partly responsible for the lack of success in some immunization experiments.

This may apply to the intraperitoneal injections of living cells in rabbits of Caillero (10) (pages 36, 48 and 50) intraperitoneal injections of living cells in guinea pigs of Ninni and Fitupaldi (96) (pages 37—38 and 48—49) intraperitoneal injections of living cells in mice of Fischer and Horthach (31) (pages 38, 49 and 50—51).

(b) All injections in successful immunization trials were given subcutaneously except for 4 of 6 injections in the experiments of Hoffmeister, Dickgesser and Göting (48) (pages 36—37 and 49) who started immunizing rabbits subcutaneously and continued by intravenous route.

(4) *Dead cells or their actions* were used either throughout the immunization or at least during its early stage in all successful experiments. The only exception is one of the experiments of Mourad and Friedman (89) (pages 38—40, 49 and 52) in which slight protection was achieved by subcutaneous injections of viable cells of *Candida*. When dead cells are used a constant production and liberation of the endotoxin-like substance by the cells contained in the vaccine is not possible, and its depressing action on nonspecific resistance is thus eliminated.

(5) Another factor necessary for a favorable outcome of immunization is a *sufficient quantity and relative ease of availability of the specific protective antigen*.

The limited experiments of Concetti (14) (page 42) and our findings (Figure 1) demonstrate that cell walls alone may protect against challenge and that other parts of the cell are ap-

parently not needed for specific protection.

Own experiments have demonstrated that only a relatively high weight of cell wall material induces specific immunity. It was shown that while 0.1 mg. and 1.0 mg. of cell walls given 3 times weekly for 3 weeks was ineffective, 5 mg. protected a significant percentage of mice. One mg. of cell walls was prepared from approximately 250 000 000 cells.

It should be noted that there was a rather marked difference between the results of immunization with merthiolate-killed and sonically vibrated cells in the experiments of Mourad and Friedman (89) (pages 38-40, 49-50 and 51) although the number of cells in both vaccines was the same and although no fraction of the cells was discarded in either vaccine. Several factors may have been responsible for the difference. One possibility is that sonic vibrations may have caused easier availability and therefore a more pronounced effect of the protective cell wall antigen. If this assumption is correct the antigen may possibly be expected to be located in an inside layer of the cell wall which may have been exposed to the tissues of the host as a result of the disintegration of the cells. Another possibility an easier availability of the endotoxin like substance and its effect on increased host resistance was discussed on pages 49-50.

Although details of clinical results in the supposedly successful vaccine treatments of Thiers, Coudert, Colomb, Fayolle and Moulin (140) (page 46) are not given it is possible that the

treatment of fungal cells with sulfonic acid by the method of Jauson and Boidé (55) may have not only destroyed the sensitizing materials, as the authors claim, but also the endotoxin-like substance. It may have, moreover dissolved the outer layer of the cell wall. This appears possible in view of the work of Mundkur (90) who has shown that boiling of yeast in dilute acid removed the outer layer of mannan with the associated protein. If the treatment of *Candida* with acid done by the French authors caused the same chemical change it may be expected that the removal of the outer mannan-protein or glucomannan-protein layer would leave the inner glucan protein layer as the active antigen in their vaccine. It was mentioned above that the same tentative conclusion about the location of the protective antigen may be reached by an evaluation of the experiment of Mourad and Friedman (89).

Vaccine therapy of animals already infected with *Candida* appears possible on the basis of the experiments of Conetti (14) (page 42) and Formina and Stepanushcheva (34) (page 42).

It has been shown already in 1904 by del Pont (16) (page 43) that passive immunization of rabbits with rabbit immune serum was possible. This finding was confirmed by Hoffmeister, Dickgiesser and Götting (48) (page 43). The size of the inoculum in their experiments may have been reduced, however by a simultaneous injection of immune serum and fungal challenge. Similar experiments of Hurd and Drake (53) (pages 43-44) were essentially unsuccessful.

Both Ata (1) (page 44) and Hasenclever and Mitchell (40) (page 44) immunized mice passively with rabbit immune serum. The former author was able to protect some animals while the experiments of the latter authors were unsuccessful.

The lack of success in some attempts to immunize passively may be due to the fact that besides protective antibodies hypersensitivity to *Candida* may have been passively transferred to immunized animals. This explanation may also apply to the report of Elinor and Byström (24) although the details of their experiments are not given. These authors stated that some mice infected with *Candida* and given anti-*Candida* serum died more rapidly than controls injected with normal serum. It is interesting in this connection that although the serum of rabbits used by Hurd and Drake (53) (pages 43-44) for passive immunization had high agglutinin titer it did not protect against challenge. As mentioned before (page 33) Kesten and Mott (61) were able to transfer sensitivity to *Monilia pilosus* passively to guinea pigs. Since Karotchkín and Luo (58) (page 38) have shown that sensitization predisposes to generalization of monilial infection it may be that spread of *Candida* infection in these experiments resulted from sensitization and was not prevented by the transfer of protective antibodies or by high agglutinin titer.

The experiments of Connor (15) (pages 44-45) who used human serum in mice and those of Fomina and Stepanishevskaya (34) (page 45) using rabbit serum in mice point to the possi-

bility that serum therapy of animals previously infected with *Candida* may be possible.

### (c) Characteristics of the Soluble

#### Extract of *Candida albicans*

Since own experiments have shown that the production of anaphylaxis and the depression or enhancement of nonspecific resistance is possible with the soluble extract of *Candida* but not with the cell wall preparation it appears that the sensitizing antigen and the endotoxin-like substance are located in the soluble cell extract. This fraction originates in the cytoplasm and possibly also in the slimy layer or capsule covering the rough outer surface of the cell wall which was demonstrated on electron microscopy by Nagata (92). Whether it contains in addition some soluble constituents of the cell wall which may have been separated from it in the course of mechanical disintegration of the cells cannot be decided without additional electron microscopic and perhaps biochemical and biophysical studies.

Because of the experimental results indicating that several apparently unrelated activities reside in the soluble extract of *Candida*, it may be fruitful to fractionate and characterize it chemically.

There is a possibility that the endotoxin-like substance may be located on or close to the surface of the cell. The fact that Kalvin (123) has prepared a toxic substance from *Candida albicans* by treating its yeast cells in the Mickle tissue disintegrator may support this possibility. Own observations revealed that only a negligible percentage of cells

parently not needed for specific protection.

Own experiments have demonstrated that only a relatively high weight of cell wall material induces specific immunity. It was shown that while 0.1 mg and 1.0 mg of cell walls given 3 times weekly for 3 weeks was ineffective, 5 mg protected a significant percentage of mice. One mg of cell walls was prepared from approximately 250 000 000 cells.

It should be noted that there was a rather marked difference between the results of immunization with merthiolate-killed and sonically vibrated cells in the experiments of Mourad and Friedman (89) (pages 38—40 49—50 and 51) although the number of cells in both vaccines was the same and although no fraction of the cells was discarded in either vaccine. Several factors may have been responsible for the difference. One possibility is that sonic vibrations may have caused easier availability and therefore a more pronounced effect of the protective cell wall antigen. If this assumption is correct the antigen may possibly be expected to be located in an inside layer of the cell wall which may have been exposed to the tissues of the host as a result of the disintegration of the cells. Another possibility an easier availability of the endotoxin like substance and its effect on increased host resistance was discussed on pages 49—50.

Although details of clinical results in the supposedly successful vaccine treatments of Thiers, Coudert, Colomb Fayolle and Moulin (140) (page 46) are not given it is possible that the

treatment of fungal cells with sulfuric acid by the method of Jausion and Boidé (55) may have not only destroyed the sensitizing materials, as the authors claim, but also the endotoxin-like substance. It may have, moreover dissolved the outer layer of the cell wall. This appears possible in view of the work of Mundkur (90) who has shown that boiling of yeast in dilute acid removed the outer layer of mannan with the associated protein. If the treatment of *Candida* with acid done by the French authors caused the same chemical change it may be expected that the removal of the outer mannan protein or glucomannan-protein layer would leave the inner glucan-protein layer as the active antigen in their vaccine. It was mentioned above that the same tentative conclusion about the location of the protective antigen may be reached by an evaluation of the experiment of Mourad and Friedman (89).

*Vaccine therapy* of animals already infected with *Candida* appears possible on the basis of the experiments of Conzetti (14) (page 42) and Fomina and Stepanushcheva (34) (page 42).

It has been shown already in 1904 by del Pont (16) (page 43) that *passive immunization* of rabbits with rabbit immune serum was possible. This finding was confirmed by Hoffmeister Dickgrasser and Götting (48) (page 43). The size of the inoculum in their experiments may have been reduced however by a simultaneous injection of immune serum and fungal challenge. Similar experiments of Hurd and Drake (53) (pages 43—44) were essentially unsuccessful.

Both Ata (1) (page 44) and Hasenclever and Mitchell (40) (page 44) immunized mice passively with rabbit immune serum. The former author was able to protect some animals while the experiments of the latter authors were unsuccessful.

The lack of success in some attempts to immunize passively may be due to the fact that besides protective antibodies hypersensitivity to *Candida* may have been passively transferred to immunized animals. This explanation may also apply to the report of Ehnov and Byström (24) although the details of their experiments are not given. These authors stated that some mice infected with *Candida* and given anti-*Candida* serum died more rapidly than controls injected with normal serum. It is interesting in this connection that although the serum of rabbits used by Hurd and Drake (53) (pages 43-44) for passive immunization had high agglutinin titer it did not protect against challenge. As mentioned before (page 53) Kesten and Mott (61) were able to transfer sensitivity to *Moussis pilosus* passively in guinea pigs. Since Kurouchkin and Lim (66) (page 33) have shown that sensitization predisposes to generalization of monilial infection it may be that spread of *Candida* infection in these experiments resulted from sensitization and was not prevented by the transfer of protective antibodies or by high agglutinin titer.

The experiments of Connor (15) (pages 44-45) who used human serum in mice and those of Fomina and Stepanishcheva (34) (page 45) using rabbit serum in mice point to the possi-

bility that serum therapy of animals previously infected with *Candida* may be possible.

### (c) Characteristics of the Soluble Extract of *Candida albicans*

Since own experiments have shown that the production of anaphylaxis and the depression or enhancement of nonspecific resistance is possible with the soluble extract of *Candida* but not with the cell wall preparation it appears that the sensitizing antigen and the endotoxin-like substance are located in the soluble cell extract. This fraction originates in the cytoplasm and possibly also in the slimy layer or capsule covering the rough outer surface of the cell wall which was demonstrated on electron microscopy by Nagata (92). Whether it contains in addition some soluble constituents of the cell wall which may have been separated from it in the course of mechanical disintegration of the cells cannot be decided without additional electron microscopic and perhaps biochemical and biophysical studies.

Because of the experimental results indicating that several apparently unrelated activities reside in the soluble extract of *Candida* it may be fruitful to fractionate and characterize it chemically.

There is a possibility that the endotoxin-like substance may be located on or close to the surface of the cell. The fact that Salvén (123) has prepared a toxic substance from *Candida albicans* by treating its yeast cells in the Mickle tissue disintegrator may support this possibility. Own observations revealed that only negligible percentage of cells



parently not needed for specific protection.

Own experiments have demonstrated that only a relatively high weight of cell wall material induces specific immunity. It was shown that while 0.1 mg and 1.0 mg of cell walls given 3 times weekly for 3 weeks was ineffective, 5 mg protected a significant percentage of mice. One mg of cell walls was prepared from approximately 250 000 000 cells.

It should be noted that there was a rather marked difference between the results of immunization with merthiolate-killed and sonically vibrated cells in the experiments of Mourad and Friedman (89) (pages 38—40 49—50 and 51) although the number of cells in both vaccines was the same and although no fraction of the cells was discarded in either vaccine. Several factors may have been responsible for the difference. One possibility is that sonic vibrations may have caused easier availability and therefore a more pronounced effect of the protective cell wall antigen. If this assumption is correct the antigen may possibly be expected to be located in an inside layer of the cell wall which may have been exposed to the tissues of the host as a result of the disintegration of the cells. Another possibility an easier availability of the endotoxin-like substance and its effect on increased host resistance, was discussed on pages 49—50.

Although details of clinical results in the supposedly successful vaccine treatments of Thiers, Coudert, Colomb Fayolle and Moulin (140) (page 46) are not given it is possible that the

treatment of fungal cells with sulfuric acid by the method of Jauson and Boidé (55) may have not only destroyed the sensitizing materials, as the authors claim, but also the endotoxin-like substance. It may have, moreover dissolved the outer layer of the cell wall. This appears possible in view of the work of Mundkur (90) who has shown that boiling of yeast in dilute acid removed the outer layer of mannan with the associated protein. If the treatment of *Candida* with acid done by the French authors caused the same chemical change it may be expected that the removal of the outer mannan-protein or glucomannan protein layer would leave the inner glucan protein layer as the active antigen in their vaccine. It was mentioned above that the same tentative conclusion about the location of the protective antigen may be reached by an evaluation of the experiment of Mourad and Friedman (89).

Vaccine therapy of animals already infected with *Candida* appears possible on the basis of the experiments of Concetti (14) (page 42) and Fomina and Stepanushcheva (34) (page 42).

It has been shown already in 1904 by del Pont (16) (page 43) that passive immunization of rabbits with rabbit immune serum was possible. This finding was confirmed by Hoffmeister Dickgeisser and Götting (48) (page 43). The size of the inoculum in their experiments may have been reduced however by a simultaneous injection of immune serum and fungal challenge. Similar experiments of Hurd and Drake (53) (pages 43—44) were essentially unsuccessful.

in own experiments, morphological and chemical studies including fractionation of the cell wall material and an investigation of immunologic and other properties of the various fractions would, therefore, appear to be fruitful steps toward deriving a suitable vaccine which would be devoid of side effects.

Since specific protection may be demonstrated with washed isolated cell walls and not with the soluble extract, it may be concluded that the protective antigen is located in the cell wall. Washed cell walls produced, however local tissue irritation in addition to protection against challenge (page 14)

It is interesting in this connection that cell walls of some other organisms are apparently also the site of protective properties. It was shown that cell wall materials from *Brucella abortus* (Marlenson, Sutfmann and Oltzki, 81) *Pasteurella pestis* (Keppae, Cocking and Smith, 59) *Bacterium tularensis* (Shepard, Ribi and Larson, 153) *Bordetella pertussis* (Munoz, Ribi and Larson, 91) *Salmonella enteritidis* (Ribi, Milner and Perrine 109) and *Histoplasma capsulatum* (Salvin and Ribi, 126) contain substances capable of specifically immunizing against infection.

Although most of the work dealing with chemical composition and electron microscopic structure of yeasts and yeast-like fungi was done with *Saccharomyces cerevisiae* some experimental results are available concerning the cell walls of the fungi of the genus *Candida*. Northcote and Horne (97) have shown that cell walls of *S. cerevisiae* are composed of protein, lipid and at least two polysaccharides, a mannan and a glucan.

They have reported that the mannan is associated with a protein.

Kessler and Nickerson (60) have found that cell wall polysaccharides of *Candida albicans* occur as protein complexes. They have recovered a glucan-protein complex and two types of glucomannan-protein complexes from walls of mechanically disrupted cells of both *S. cerevisiae* and *C. albicans* and have shown a similarity of the fractions derived from the two organisms. The glucan-protein complex of *C. albicans* contained only around 1 % protein nitrogen while one of the glucomannan-protein complexes had a protein nitrogen of 3-4 %. The glucomannan-protein complexes were extracted with dilute potassium hydroxide and the glucan-protein complex constituted the insoluble residue. Bishop, Blank and Gardner (3) believe, however that the glucomannans reported were mixtures of glucan and mannan. Houwink and Kreger (51) reported that a small amount of chitin is also present in the cell walls of *Candida tropicalis*. This was confirmed by the experiments of Bishop, Blank and Gardner (3) who examined the details of the chemical structure of the cell wall polysaccharides of *C. albicans*. Kessler and Nickerson (60) found about 1 % of lipid in the cell walls of *C. albicans*.

It was mentioned in a previous chapter that the existence of at least two layers may be postulated in the cell walls of *C. albicans* in order to interpret the apparent increased antigenicity in the vaccines prepared from disrupted organisms as compared to vaccines from intact cells in the experiments performed

was disintegrated by this apparatus even after a treatment lasting several hours. It is possible, therefore, that the toxic substance in Salvin's experiments may have been released by this treatment from the cell surface. Roth and Murphy (115) released a toxic substance by ultrasonic vibration of *Candida* cells most of which remained intact. They believed that the active substance from the cell free filtrate which was lethal to mice treated with chlortetracycline originated on the surface of the cells. Isenberg, Allerhand and Berkman (54) extracted with ethanol-ethyl ether or phenol a toxic substance from intact cells of *C. albicans* which according to them originated on the cell surface.

The endotoxin like substance may possibly be located in the layer of slime seen on electron microscopy by Nagata (92). Negroni (93) observed a polysaccharide capsule in *C. albicans* and reported that it was not easily separated by shaking of yeast cells with glass beads. It is possible that the fact that the toxicity of Salvin's extract was manifested only after the addition of an adjuvant and that the preparation of Roth and Murphy (115) was toxic only in chlortetracycline treated mice may be explained by incomplete separation of this slime layer by mechanical means. A relatively high toxicity of the own preparation may perhaps be explained by a very thorough mechanical treatment. The question of the localization of the endotoxin-like substance may possibly be clarified on electron microscopy of cells treated by the above mentioned methods.

Since none of the toxic extracts have

been chemically characterized it is impossible to decide whether or not these variously prepared substances are identical. If a similarity exists in chemical composition of endotoxins of gram-negative bacterial organisms and *Candida* this toxic substance may be a lipopoly saccharide or possibly the lipid A fraction thereof according to Westphal et al. (147).

It appears possible that at least most of the sensitizing properties are located in another fraction or fractions, probably originating in the cytoplasm and containing protein.

One of the main conclusions of the present paper which may be reached on the basis of own experimental studies and of a critical analysis of the literature is that an effective vaccine against moniliasis should not contain any portion of the soluble extract since it contains the endotoxin like substance and most or all of the fraction or fractions producing sensitization.

#### (d) Characteristics of Cell Walls

An ideal protective vaccine which would have to be nonviable, should, if possible contain a single antigenic component so that no local or general side effects would result from its administration. It is possible that a polysaccharidic antigen from the cell walls may fulfill these requirements. Findings and speculations which may be pertinent in the search for such an antigenic component in the cell wall will, therefore be briefly discussed.

Since possibly several unrelated immunologic and other properties may reside in the cell wall preparation used

the most likely site of the protective antigen, and that the irritating property may reside in the outer soluble layer composed of the glucomannan-protein complex.

#### (c) *Role of Endotoxins in Nonspecific Resistance*

Instances of a change in host resistance to infections following the administration of various substances have been frequently described in the literature since the end of the last century (10<sup>2</sup>). In many of these findings the active components have been shown to be endotoxins of gram-negative bacteria (8, 62, 69). The infections affected by this action are those due to gram-negative bacteria (8, 62, 69) mycobacteria (19) staphylococci (19) streptococci (62), and some viruses (64-65, 83, 94). It was also found that substances of microbial origin other than gram-negative endotoxins, such as pertussis vaccine (19) and killed *Mycobacterium tuberculosis* or its extracts (20) pneumococcus polysaccharides (62) and symosan (62) are capable of modifying nonspecific host resistance. Substances not derived from microorganisms, such as mucin (62) colloidal sulfur (62) and sterile milk (8) have also been found to have some resistance-influencing activity against experimental infections.

The response to administration of these substances was shown to be a biphasic change in resistance. An injection of an endotoxin is followed by several hours to several days of decreased resistance after which resistance to bacterial challenge is increased for several days to several weeks. The relative and

absolute duration of the two phases appears to be dependent to a certain degree on the size of the dose of the substance used to modify the infection (19, 52, 69). A large dose of an endotoxin is followed by a relatively long phase of decreased resistance and a prolonged phase of increased resistance. Injections of these materials given simultaneously with or after bacterial infections markedly shortened the life of infected animals. Chronic bacterial infections may be converted into acute, at times fatal diseases under these conditions (127).

It is not in the scope of this paper to review the possible mechanisms by which endotoxins affect host reaction to infections. At present the knowledge of these mechanisms is incomplete. Among the many systems suggested to be operative in the production of the changes in host resistance the following may be mentioned: the reticuloendothelial system (7, 52) mobile phagocytes (12) bactericidal serum factors (32, 116) the properdin system (70) serum lysozyme (49) activation of fibrinolysis (50) inhibition of allergic reactions (84) and stimulation of antibody production (148).

The resistance-influencing substance of the soluble extract from the yeast cells of *Candida albicans* is referred to in this paper as endotoxin-like since it was shown in our unpublished experiments to exhibit, besides the resistance-influencing properties reported here several other properties similar to those of the endotoxins of gram-negative bacteria. It was found e. g. that the soluble extract of *Candida* caused general

by Mourad and Friedman (89). It was suggested that perhaps mechanical disintegration may have exposed an inside layer of the cell wall to the host tissues and made thus more easily available to the animals the protective antigen which may possibly be located in this layer (page 52).

Indeed, Yoshizawa (154) has shown that *C. albicans* possesses a cell wall about 150 m $\mu$  thick consisting of an outer relatively electron-dense and an inner less dense layer. This finding was confirmed by Nagata (92) who also reported that the rough outer surface of the external layer is covered with a layer of slime. Similar morphology of the cell wall of *C. albicans* was reported by Gale (37a) who found that the outer relatively electron-dense layer was about 40 m $\mu$  in thickness and that the inner electron thin area was about 200 m $\mu$  thick. The outer surface of the cell wall shown in his electron micrographs is rough.

This arrangement appears similar to that of the cell wall of *S. cerevisiae* which has been studied by several authors. Northcote and Horne (97) have shown that the cell walls of *S. cerevisiae* consist of at least two membranes one of which is made up of a glucan component. Mundkur (90) using special cytochemical methods for polysaccharides in electron microscopy of frozen dried yeast cells confirmed the existence of two layers in the cell walls of *S. cerevisiae*. He showed that the outer one which had a higher electron density was composed of mannan with an associated protein. The inner layer was shown to be comprised of glucan. Boiling in

dilute acid removed mannan with the associated protein. Eddy (23) also examined the topographic localization of the two polysaccharidic components of yeast cell walls. He found that the mannan protein complex was released from the cell walls of *S. cerevisiae* by the treatment with papain. He suggested that this complex is located at the surface of the cell wall. Houwink and Kreger (51) found a network of very thin fibrils in the walls of *C. tropicalis*. Nickerson and Falcone (95) reported that the glucan component of yeast cell wall possessed the fibrillar structure.

These findings suggest that *Candida* has a cell wall consisting of two layers. The outer layer having a high electron density on electron microscopy is composed of a probably globular soluble mannan protein or glucomannan-protein complex or complexes. The inner layer of low electron density is comprised of a glucan protein complex which has probably a microfibrillar structure. It is relatively insoluble and constitutes the residue after extraction of the glucomannan-protein complexes with dilute potassium hydroxide. Extraction of the outer layer may also be possible with papain or boiling with dilute acid. The inner layer contains less protein than the outer layer.

It is quite possible that the protective antigen and the substance containing the irritating property may not reside in the same morphological or chemical component of the cell wall. In view of the mentioned facts and speculations a working hypothesis may be established that the inside layer containing the insoluble glucan-protein complex may be

Ravin, Rowley Jenkins and Fine (107) have shown by studies with labeled microorganisms that the source of the circulating endotoxin are the organisms constituting the normal gastrointestinal flora, and that this endotoxin is continuously absorbed from the intestine. It is reasonable to assume that relatively more endotoxin is suddenly liberated and absorbed in the course of antibiotic treatment as a result of massive destruction of enteric organisms which in most instances is unintentional since such therapy is usually not directed against them. Orth and Rumiczka (98) and Rumiczka (119) have shown by electron microscopy that bacterial organisms, e. g. *Salmonella typhosa* are disrupted by antibiotics and their cytoplasmic contents are leaving the bacterial cells. Molaret (88) has demonstrated that the effect of released endotoxin during antibiotic therapy which is due to a massive destruction of microorganisms, is similar to the Herzfelmer reaction. He has observed that guinea pigs infected with *Salmonella paratyphi B* (*S. Schottmülleri*) when treated with large doses of chloramphenicol, exhibited histologic changes in organs typical of those seen in animals injected with endotoxin. Some of the guinea pigs died as a result of the effect of endotoxin.

Fine, Rutenburg and Schweinburg (28) demonstrated death of the majority of rabbits with coliform intestinal flora given a sublethal dose of thorotrast, intended to produce a blockade of the reticuloendothelial system, and administered polymyxin or neomycin either in one dose several hours after the blockade or in several doses given over 3-4 days

before and in one dose after the blockade. Most of coliform-free rabbits treated the same way remained alive. Bacitracin given instead of either polymyxin or neomycin was harmless. The authors interpreted the death of polymyxin and neomycin-treated rabbits by a sudden and substantial increase in the amount of endotoxin entering the circulation as a result of the administration of the antibiotic. Death occurred because the blockaded reticuloendothelial system was unable to cope with the increased amount of endotoxin absorbed into the circulation. They believed that when there is an increase in the rate of production of the endotoxin in the intestine there is also an increased amount absorbed. No endotoxemia or death could be produced by bacitracin in rabbits with coliform flora, apparently since this flora is not sensitive to this antibiotic. Neomycin or polymyxin in coliform-free animals caused only a few deaths since the size of the pool of endotoxin in the intestine was reduced. The authors attributed the death of the few animals to the relativity of the term coliform-free.

It is obvious that besides these toxic, shock-producing and lethal effects the endotoxins which had been released and absorbed as a result of antibiotic treatment may exhibit some of their other properties, among them the effect on the nonspecific resistance to infection. Since it was shown in experiments reported in this paper that *Candida* infections are enhanced by bacterial endotoxin injected after an infecting dose of *Candida* had been given (Figures 7 and 8) it appears highly probable that the

toxicity and was lethal to mice when injected intravenously or intraperitoneally in suitable doses. Its hematologic effects in mice included the production of leukocytosis with relative lymphopenia. A lymphocytosis was produced after repeated administration. It was also capable of prolonging life of mice with transmitted L1210 leukemia and with the spontaneous leukemia in AKR mice. Generalized loss of hair was observed after repeated administration.

The experiments reported in this paper demonstrate that modification of *Candida* and *Salmonella* infections in mice by endotoxins of gram negative bacteria and by the endotoxin-like substance of *C. albicans* is comparable, both qualitatively and quantitatively.

It is not known whether the chemical nature and mode of action on non-specific resistance of microbial substances other than gram negative endotoxins are similar to those of the endotoxins of gram negative bacteria. The same applies, of course, to the endotoxin-like substance of *Candida*.

#### (f) Possible Role of Bacterial Resistance Influencing Substances in Pathogenesis of Moniliasis

The effects of the endotoxins of gram-negative bacterial organisms on *Candida* have received only little attention. Gale and Sandoval (37) who injected mice with the endotoxin of *Escherichia coli* 30 minutes after they had been infected with *Candida albicans* found that their survival time was shortened. Hasenclever and Mitchell (41) have reported partial protection against a *Candida* infection in mice pretreated with *Sal-*

*monella enteritidis* or *S. typhosa* endotoxin.

The fact that endotoxins of gram-negative organisms are capable of enhancing or depressing the spread of *Candida* in experimental animals, depending on the time of their administration, may be helpful in explaining some aspects of the pathogenicity of this yeast like organism.

(1) Among the unsolved problems which may be seen in a new light on the basis of the findings reported here which demonstrate that bacterial endotoxins affect resistance to *Candida* the most important one appears to be the question why *Candida* infections are enhanced in patients on antibiotic treatment or why persons who are asymptomatic carriers of *Candida* in the intestinal tract develop clinical moniliasis during or following antibiotic therapy.

It is possible that the administration of an antibiotic, especially one with a "broad spectrum" effective also against gram-negative organisms, destroys much of the normal bacterial flora of the body mainly of the intestinal tract, which consists to a large extent of gram-negative endotoxin-producing organisms. By the sudden destruction of these organisms endotoxins may be liberated which may enhance the growth of microorganisms which are susceptible to the nonspecific resistance-influencing action of endotoxins and which are resistant to the administered antibiotic. Among them *C. albicans* is one of the potentially pathogenic microorganisms commonly present in the intestinal tract and not susceptible to the action of the usually administered antibiotics.

Ramm, Rowley Jenkins and Fine (107) have shown by studies with labeled microorganisms that the source of the circulating endotoxin are the organisms constituting the normal gastrointestinal flora, and that this endotoxin is continuously absorbed from the intestine. It is reasonable to assume that relatively more endotoxin is suddenly liberated and absorbed in the course of antibiotic treatment as a result of massive destruction of enteric organisms which in most instances is unintentional since such therapy is usually not directed against them. Orth and Ruzicka (98) and Ruzicka (119) have shown by electron microscopy that bacterial organisms, e. g. *Salmonella typhosa*, are disrupted by antibiotics and their cytoplasmic contents are leaving the bacterial cells. Mollaret (88) has demonstrated that the effect of released endotoxin during antibiotic therapy which is due to massive destruction of microorganisms, is similar to the Herrnhelmer reaction. H. has observed that guinea pigs infected with *Salmonella paratyphi B* (*S. S. disenteriae*) when treated with large doses of chloramphenicol, exhibited histologic changes in organs typical of those seen in animals injected with endotoxin. Some of the guinea pigs died as result of the effect of endotoxin.

Five Rutenburg and Schweinburg (28) demonstrated death of the majority of rabbits with coliform intestinal flora given sublethal dose of thorotrast intended to produce a blockade of the reticuloendothelial system, and administered polymyxin or neomycin either in one dose several hours after the blockade or in several doses given over 3-4 day

before and in one dose after the blockade. Most of coliform-free rabbits treated the same way remained alive. Bacitracin given instead of either polymyxin or neomycin was harmless. The authors interpreted the death of polymyxin and neomycin-treated rabbits by a sudden and substantial increase in the amount of endotoxin entering the circulation as a result of the administration of the antibiotic. Death occurred because the blocked reticuloendothelial system was unable to cope with the increased amount of endotoxin absorbed into the circulation. They believed that when there is an increase in the rate of production of the endotoxin in the intestine there is also an increased amount absorbed. No endotoxemia or death could be produced by bacitracin in rabbits with coliform flora, apparently since this flora is not sensitive to this antibiotic. Neomycin or polymyxin in coliform-free animals caused only a few deaths since the size of the pool of endotoxin in the intestine was reduced. The authors attributed the death of the few animals to the relativity of the term coliform-free.

It is obvious that besides these toxic, shock-producing and lethal effects the endotoxins which had been released and absorbed as a result of antibiotic treatment may exhibit some of their other properties, among them the effect on the nonspecific resistance to infection. Since it was shown in experiments reported in this paper that *Candida* infections are enhanced by bacterial endotoxin injected after an infecting dose of *Candida* had been given (Figures 7 and 8) it appears highly probable that the



sudden release of endotoxin from intestinal flora is responsible for the enhancement of *Candida* infections during antibiotic treatment.

One may further speculate whether another known property of the endotoxins, viz. their beneficial effect on survival after radiation observed by Smith, Alderman and Gillespie (136) may not take place in irradiated animals subjected to antibiotic treatment, as a result of the release and absorption of endotoxin from the intestinal flora caused by antibiotics. This assumption would explain more completely the experiments of Miller Hammond, Tompkins and Shorter (87) who were able to reduce mortality rates in mice exposed to x radiation by the administration of antibiotics, especially those effective against the members of the normal intestinal flora. Suppression of secondary infections in irradiated animals was apparently not the full explanation of their experiments.

A series of experiments with germ-free animals is planned to confirm the assumption that endotoxins released from intestinal flora by antibiotics enhance the growth of *Candida*. It is expected that antibiotics will not enhance the growth of *Candida* in animals which are otherwise free of microorganisms.

If this supposition is correct measures directed toward inactivation of endotoxins may be helpful in the prevention and treatment of moniliasis enhanced by antibiotic therapy. Although the mechanisms of inactivation of endotoxins are not clearly understood some facts have been established.

Several workers have observed the

presence, in mammalian blood (43 47 195) liver (141) and spleen (118) of factors inactivating endotoxin, which are presumably of enzymatic nature. In the experience of Rutenburg, Smith, Rutenburg and Fine (118) a splenic extract was much more effective than either liver extracts or serum. Although these factors have not been tested for their possible property of inactivating the modifying influence of endotoxins upon resistance to infections, other endotoxin-inactivating properties of these factors have been demonstrated. It was shown that the in vitro incubation of endotoxin with these substances results in an inactivation of lethality for rodents (47) tumor necrotization (71) induction of the Shwartzman phenomenon (71) pyrogenicity (43 153) and elevation of leucocytes (43). The protective effect of the human plasma was most marked in Cohn's fractions III and IV (47 153).

Rutenburg, Schweinsburg and Fine (117) have demonstrated that plasma in combination with reticuloendothelial macrophages is effective in the in vitro inactivation of endotoxin.

Furthermore, Takeda, Miura, Suzuki and Kasai (139) have shown that adenosine triphosphate administered intravenously in mice inactivated the lethal property and disturbances in phosphorus and lactic acid metabolism induced by endotoxins.

Janoff and Zweisach (53) have shown that after incubation of endotoxin with iron its tissue necrotizing properties in rabbits were decreased.

It may be rewarding to test the effectiveness of these factors for the in-

activation of the nonspecific stimulation of growth of *Candida* by gram-negative endotoxins. These methods may serve as a basis for a future clinical application. It is conceivable e. g., that an enzyme preparation, when isolated from animal tissues in a more purified form, may be used therapeutically. The mentioned findings may also raise the question whether patients with moniliasis enhanced by antibiotics may not benefit from blood or plasma transfusions because of the contents of an endotoxin-neutralizing substance in the blood.

(2) The development of moniliasis, at times fatal, has been described following immunization either against diphtheria, pertussis and tetanus (38) with BCG (106, 138) or with a small pox vaccine (4, 58). The enhancement of the growth of *Candida* after immunization against diphtheria, pertussis and tetanus may have been due to the injection of the pertussis vaccine with known resistance-influencing properties. (19) Tubercle bacilli have been shown to enhance certain bacterial infections in a similar way as the endotoxins of gram-negative bacilli (20). Dead tubercle bacilli are also used as adjuvants in biological experiments and they were found to be capable of enhancing *Candida* infections (123). Whether or not the virus of cowpox contains substances capable of enhancing infections in a similar way as the bacterial endotoxins is not known.

(3) It is not clear whether the mode of action as well as the methods of inactivation of resistance-influencing substances of microbial origin other than gram-negative endotoxins, such as those

contained in the pertussis vaccine or tubercle bacilli, are identical with those applicable to the endotoxins of gram-negative bacteria. Since these substances may be responsible for the enhancement of *Candida* infections in the above described situations it may be worth while to test the earlier mentioned (pages 60—61) methods for the inactivation of endotoxins also against them.

(4) The variability of the intestinal flora of experimental animals (128) was thought to be a reason for differences in the susceptibility to bacterial infections (22). This susceptibility may be, at least partly related to the size of the intraintestinal pool of available endotoxins and resulting different opportunities to absorb endotoxins from the intestinal flora (28). It is also perhaps one of the factors responsible for the marked variations in the resistance to a standardized *Candida* infection from one animal to another and, usually even to a larger extent, from one colony of animals to another which has been seen in many own experiments. It is possible that the differences in susceptibility to infections which might have been thought to be genetically determined (36) in reality are due to differences in intestinal flora (21).

(g) *Possible Role of Resistance-Influencing Substance of Candida in Pathogenesis of Moniliasis*

It appears possible from the reviewed literature and from own experiments that one of the principal reasons for multiplication of *Candida* in systemic moniliasis may be the constant liberation of the endotoxin-like substance

sudden release of endotoxin from intestinal flora is responsible for the enhancement of *Candida* infections during antibiotic treatment.

One may further speculate whether another known property of the endotoxins, viz. their beneficial effect on survival after radiation, observed by Smith, Alderman and Gillespie (136) may not take place in irradiated animals subjected to antibiotic treatment, as a result of the release and absorption of endotoxin from the intestinal flora caused by antibiotics. This assumption would explain more completely the experiments of Miller Hammond, Tompkins and Shorter (87) who were able to reduce mortality rates in mice exposed to  $\gamma$  radiation by the administration of antibiotics, especially those effective against the members of the normal intestinal flora. Suppression of secondary infections in irradiated animals was apparently not the full explanation of their experiments.

A series of experiments with germ-free animals is planned to confirm the assumption that endotoxins released from intestinal flora by antibiotics enhance the growth of *Candida*. It is expected that antibiotics will not enhance the growth of *Candida* in animals which are otherwise free of microorganisms.

If this supposition is correct measures directed toward inactivation of endotoxins may be helpful in the prevention and treatment of moniliasis enhanced by antibiotic therapy. Although the mechanisms of inactivation of endotoxins are not clearly understood, some facts have been established.

Several workers have observed the

presence, in mammalian blood (43 47 135) liver (141) and spleen (118) of factors inactivating endotoxin, which are presumably of enzymatic nature. In the experience of Rutenburg, Smith, Rutenburg and Fine (118) a splenic extract was much more effective than either liver extracts or serum. Although these factors have not been tested for their possible property of inactivating the modifying influence of endotoxins upon resistance to infections, other endotoxin inactivating properties of these factors have been demonstrated. It was shown that the in vitro incubation of endotoxin with these substances results in an inactivation of lethality for rodents (47) tumor necrotization (71) induction of the Shwartzman phenomenon (71) pyrogenicity (43 153) and elevation of leucocytes (43). The protective effect of the human plasma was most marked in Cohn's fractions III and IV (47 153).

Rutenburg, Schweinburg and Fine (117) have demonstrated that plasma in combination with reticuloendothelial macrophages is effective in the in vitro inactivation of endotoxin.

Furthermore, Takeda, Miura, Suzuki and Kasai (139) have shown that adenosine triphosphate administered intravenously in mice inactivated the lethal property and disturbances in phosphorus and lactic acid metabolism induced by endotoxins.

Janoff and Zweifach (55) have shown that after incubation of endotoxin with iron its tissue necrotizing properties in rabbits were decreased.

It may be rewarding to test the effectiveness of these factors for the in-

they challenged them with *C. albicans*.

Hedgerock (42) used nonviable fungal vaccines to enhance resistance to tuberculous in mice. All vaccines were prepared by killing the fungi with ethylene oxide. A partial protection against tuberculous infection was observed when the interval between intraperitoneal injection of a vaccine prepared from the mycelial phase of *H. capsulatum* and intravenous challenge was 7 days. Very little resistance was seen when the interval was 29 days. No change in the course of the tuberculous infection was observed when the yeast phase of *H. capsulatum* was used instead of the mycelial phase. Vaccines prepared from the mycelial phase of *Blastomyces dermatitidis* yeast cells of *Cryptococcus neoformans* yeast cells or mycelia of *Sporotrichum* *S. henrii* given 13 days prior to infection with *Mycobacterium tuberculosis* also enhanced resistance of mice.

The following experiments of Salvin (124) strongly suggest the possibility of an endotoxin-like substance in *H. capsulatum* and its effect on nonspecific resistance. I. a group of mice injected intraperitoneally with  $10^6$  cells of *H. capsulatum* in 5 % mucin 86 % died within 21 days. Of those dying in the first 3 weeks 73 % succumbed between the 4th and the 10th day after the injection but only a few died within 48 hours.

Another group of mice was injected intraperitoneally first with  $10^6$  cells of *Hist. plasma* and 2 weeks later with  $10^6$  cells of *Hist. plasma* in mucin intraperitoneally. Sixty-one per cent of them died during the first 3 weeks after the

second injection. Of these 90 % died during the first 48 hours. When the size of the first injection was increased to  $10^8$  cells a 4- to 5-day interval between the two injections was sufficient to produce a high percentage of early deaths.

In another experiment acetone-dried cells of *H. capsulatum* were injected intraperitoneally instead of the first injection of living cells, and were followed by  $10^6$  living cells 2 weeks later. The number of early deaths was not higher than among the controls, but a resistance to lethal challenge developed in pretreated mice.

Another group of mice was pretreated with  $10^6$  cells of *Histoplasma* and challenged with acetone-dried cells in mucin 2 weeks later. Fifty three percent of them died within 48 hours after the second injection.

Salvin interpreted the early deaths as being due to hypersensitivity. No adequate explanation may be found, however on this basis for the absence of early deaths in mice pretreated with dead cells.

On the basis of these experimental results one may postulate that *Histoplasma*, similarly as *Candida* may contain an endotoxin-like substance effective in enhancing or depressing nonspecific resistance. In the first experiment the endotoxin-like substance constantly liberated by the cells administered in the first injection may have enhanced the growth of cells in the second injection. With a relatively small primary inoculation ( $10^6$  cells) it took apparently several days for the inoculum to multi-

contained in the soluble fraction of the fungus. This substance may keep enhancing the growth of the fungus and nullify the effect of the specific protective antigen of the cell walls. The same may be true of other systemic mycoses, such as histoplasmosis. The fact that cases of wide-spread bloodborne moniliasis as well as those with extensive gastrointestinal localization can be influenced by the present methods of treatment only with the utmost difficulty may possibly be explained, at least partly by this assumption.

Supporting this supposition is an own experiment in which an injection of the soluble extract of *Candida* given to mice 3 hours after an infection with *Candida* resulted in a shortened survival time (Figure 9). The same is true of the experiments of Ninu and Fittipaldi (96) in which guinea pigs were killed by living *Candida* cells injected repeatedly in several small doses the total of which was smaller than one single sublethal dose (page 28).

Provided the mechanisms of inactivation of the endotoxin-like substances of fungi are the same as those effective against endotoxins of gram-negative bacteria, which were mentioned in the preceding chapter the experimental results concerning the inactivation of the latter may be applied in counteracting the effect of the former.

#### (h) Applicability of Immunological Findings in Moniliasis to Other Fungal Diseases

It is probable that immunological mechanisms similar to those shown here to

be operative in moniliasis are at play in other fungus diseases.

(i) The effect of endotoxins of gram-negative bacteria on a fungus other than *Candida* was demonstrated in the experiments of Louria (77) who has shown that resistance to *Cryptococcus neoformans* can be increased by a previous injection of a *Salmonella* endotoxin.

(ii) The localization of protective properties in the cell wall was reported by Salvin (124) for *Huttoplasma capsulatum*.

(iii) That cytoplasm may be the site of sensitizing substances in *H. capsulatum* may be suspected on the basis of other experiments of Salvin (124a) who increased susceptibility to growth of *H. capsulatum* in mice injected with leukocytes transferred from mice which had been treated with viable or dead whole cells of *Huttoplasma* or a cytoplasmic fraction thereof but not from those which had been treated with cell walls. It is possible that passive transfer of hypersensitivity by leukocytes induced an increased susceptibility to *H. capsulatum*.

(iv) The following experiments suggest the possibility of the presence of endotoxin-like substances in fungi other than *Candida*. Hasenclever and Mitchell (40) (page 19) injected dead disintegrated spherules of *Coccidioides immitis* 6 days before challenge with *Candida albicans* and found that the life of the pretreated mice was somewhat prolonged. In another set of experiments the same authors prolonged life of mice slightly by an injection of viable cells of *H. capsulatum*, *Cryptococcus neoformans*, *Coccidioides immitis* or *Aspergillus fumigatus* given 6 days before

they challenged them with *C. albis* var.

Hedgcock (42) used nonviable fungal vaccines to enhance resistance to tuberculous in mice. All vaccines were prepared by killing the fungi with ethylene oxide. A partial protection against tuberculous infection was observed when the interval between intraperitoneal injection of a vaccine prepared from the mycelial phase of *H. capsulatum* and intravenous challenge was 7 days. Very little resistance was seen when the interval was 29 days. No change in the course of the tuberculous infection was observed when the yeast phase of *H. capsulatum* was used instead of the mycelial phase. Vaccines prepared from the mycelial phase of *Blastomyces dermatitidis* yeast cells of *Cryptococcus neoformans* yeast cells or mycelia of *Sporotrichum Schenckii* given 13 days prior to infection with *Mycobacterium tuberculosis* also enhanced resistance of mice.

The following experiments of Salvin (124) strongly suggest the possibility of an endotoxin-like substance in *H. capsulatum* and its effect on nonspecific resistance. In a group of mice injected intraperitoneally with  $10^8$  cells of *H. capsulatum* in 5% mucin 86% died within 21 days. Of those dying in the first 3 weeks 73% succumbed between the 4th and the 10th day after the injection but only a few died within 48 hours.

Another group of mice was injected intraperitoneally first with  $10^8$  cells of *Histoplasma* and 2 weeks later with  $10^8$  cells of *Histoplasma* in mucin intraperitoneally. Sixty-one per cent of them died during the first 3 weeks after the

second injection. Of these 90% died during the first 48 hours. When the size of the first injection was increased to  $10^8$  cells a 4- to 5-day interval between the two injections was sufficient to produce a high percentage of early deaths.

In another experiment acetone-dried cells of *H. capsulatum* were injected intraperitoneally instead of the first injection of living cells, and were followed by  $10^8$  living cells 2 weeks later. The number of early deaths was not higher than among the controls, but a resistance to lethal challenge developed in pretreated mice.

Another group of mice was pretreated with  $10^8$  cells of *Histoplasma* and challenged with acetone-dried cells in mucin 2 weeks later. Fifty-three per cent of them died within 48 hours after the second injection.

Salvin interpreted the early deaths as being due to hypersensitivity. No adequate explanation may be found, however on this basis for the absence of early deaths in mice pretreated with dead cells.

On the basis of these experimental results one may postulate that *Histoplasma*, similarly as *Candida* may contain an endotoxin-like substance effective in enhancing or depressing nonspecific resistance. In the first experiment the endotoxin-like substance constantly liberated by the cells administered in the first injection may have enhanced the growth of cells in the second injection. With a relatively small primary inoculation ( $10^8$  cells) it took apparently several days for the inoculum to multi-

contained in the soluble fraction of the fungus. This substance may keep enhancing the growth of the fungus and nullify the effect of the specific protective antigen of the cell walls. The same may be true of other systemic mycoses, such as histoplasmosis. The fact that cases of wide-spread bloodborne moniliasis as well as those with extensive gastrointestinal localization can be influenced by the present methods of treatment only with the utmost difficulty may possibly be explained at least partly by this assumption.

Supporting this supposition is an own experiment in which an injection of the soluble extract of *Candida* given to mice 3 hours after an infection with *Candida* resulted in a shortened survival time (Figure 9). The same is true of the experiments of Ninni and Fittipaldi (96) in which guinea pigs were killed by living *Candida* cells injected repeatedly in several small doses the total of which was smaller than one single sublethal dose (page 28).

Provided the mechanisms of inactivation of the endotoxin-like substances of fungi are the same as those effective against endotoxins of gram-negative bacteria, which were mentioned in the preceding chapter the experimental results concerning the inactivation of the latter may be applied in counteracting the effect of the former

(h) *Applicability of Immunological Findings in Moniliasis to Other Fungus Diseases*

It is possible that immunological mechanisms similar to those shown here to

be operative in moniliasis are at play in other fungus diseases.

(i) The effect of endotoxins of gram negative bacteria on a fungus other than *Candida* was demonstrated in the experiments of Lounia (77) who has shown that resistance to *Cryptococcus neoformans* can be increased by a previous injection of a *Salmonella* endotoxin.

(ii) The localization of protective properties in the cell wall was reported by Salvin (124) for *Histoplasma capsulatum*

(iii) That cytoplasm may be the site of sensitizing substances in *H. capsulatum* may be suspected on the basis of other experiments of Salvin (124a) who increased susceptibility to growth of *H. capsulatum* in mice injected with leukocytes transferred from mice which had been treated with viable or dead whole cells of *Histoplasma* or a cytoplasmic fraction thereof but not from those which had been treated with cell walls. It is possible that passive transfer of hypersensitivity by leukocytes induced an increased susceptibility to *H. capsulatum*.

(iv) The following experiments suggest the possibility of the presence of endotoxin-like substances in fungi other than *Candida*. Hasenclever and Mitchell (40) (page 19) injected dead disintegrated spherules of *Coccidioides immitis* 6 days before challenge with *Candida albicans* and found that the life of the pretreated mice was somewhat prolonged. In another set of experiments the same authors prolonged life of mice slightly by an injection of viable cells of *H. capsulatum*, *Cryptococcus neoformans*, *Coccidioides immitis* or *Aspergillus fumigatus* given 6 days before

they challenged them with *C. albicans*.

Hedgecock (42) used nonviable fungal vaccines to enhance resistance to tuberculous infection in mice. All vaccines were prepared by killing the fungi with ethylene oxide. A partial protection against tuberculous infection was observed when the interval between intraperitoneal injection of a vaccine prepared from the mycelial phase of *H. capsulatum* and intravenous challenge was 7 days. Very little resistance was seen when the interval was 29 days. No change in the course of the tuberculous infection was observed when the yeast phase of *H. capsulatum* was used instead of the mycelial phase. Vaccines prepared from the mycelial phase of *Blastomyces dermatitidis* yeast cells of *Cryptococcus neoformans* yeast cells or mycelia of *Sporotrichum Schenckii* given 13 days prior to infection with *Mycobacterium tuberculosis* also enhanced resistance of mice.

The following experiments of Salvin (124) strongly suggest the possibility of an endotoxin-like substance in *H. capsulatum* and its effect on nonspecific resistance. In a group of mice injected intraperitoneally with  $10^6$  cells of *H. capsulatum* in 5% mucin 86% died within 21 days. Of those dying in the first 3 weeks 73% succumbed between the 4th and the 10th day after the injection but only a few died within 48 hours.

Another group of mice was injected intraperitoneally first with  $10^6$  cells of *Histoplasma* and 2 weeks later with  $10^6$  cells of *Histoplasma* in mucin intraperitoneally. Sixty-one per cent of them died during the first 3 weeks after the

second injection. Of these 90% died during the first 48 hours. When the size of the first injection was increased to  $10^8$  cells a 4- to 5-day interval between the two injections was sufficient to produce a high percentage of early deaths.

In another experiment acetone-dried cells of *H. capsulatum* were injected intraperitoneally instead of the first injection of living cells, and were followed by  $10^6$  living cells 2 weeks later. The number of early deaths was not higher than among the controls, but a resistance to lethal challenge developed in pretreated mice.

Another group of mice was pretreated with  $10^6$  cells of *Histoplasma* and challenged with acetone-dried cells in mucin 2 weeks later. Fifty-three per cent of them died within 48 hours after the second injection.

Salvin interpreted the early deaths as being due to hypersensitivity. No adequate explanation may be found, however, on this basis for the absence of early deaths in mice pretreated with dead cells.

On the basis of these experimental results one may postulate that *Histoplasma* similarly as *Candida*, may contain an endotoxin-like substance effective in enhancing or depressing nonspecific resistance. In the first experiment the endotoxin-like substance constantly liberated by the cells administered in the first injection may have enhanced the growth of cells in the second injection. With a relatively small primary inoculation ( $10^6$  cells) it took apparently several days for the inoculum to multi-



ply sufficiently to produce enough of the endotoxin like substance to enhance the growth of cells in the secondary injection. With a larger inoculum ( $10^6$  cells) enough endotoxin like substance was apparently ready 4—5 days later to enhance the growth of the challenge dose.

When acetone-dried cells were used for the first injection there were no living cells present to liberate constantly the substance affecting nonspecific resistance. No early deaths took place therefore. An increase in the resistance to lethal challenge was observed, however. This may have been due partly to the development of specific immunity and if one postulates the existence of an endotoxin like substance in *H. capsulatum* partly due to the enhancement of nonspecific resistance by this substance contained in the dead cells. A similar increase in resistance was produced by the endotoxin like substance of *Candida albicans* (Figures 2 3 4 and 5).

Enhancement of the infection took place when dead cells were injected 2 weeks after the living organisms. If the situation in histoplasmosis is similar to that in moniliasis an endotoxin-like substance injected after challenge will enhance the growth of organisms contained in the challenge dose (Figures 7 8 and 9).

(v) *Conclusions.* Although the evidence of the presence and localization of the various immunologically active fractions in fungi other than *Candida* is fragmentary several facts may nevertheless be stated and some tentative suggestions and conclusions made on the basis of the foregoing review

(1) *Histoplasma capsulatum* (a) appears to possess an endotoxin-like substance, (b) its protective antigen may be located in the cell wall, and (c) its cytoplasm may be the site of sensitizing substances. If these findings are confirmed a far reaching similarity of these features with *Candida* would be demonstrated. The working hypothesis expressed for the localization of the protective antigen of *Candida* (pages 56—57) and the tentative criteria for the preparation of an anti-*Candida* vaccine mentioned on page 54 may then perhaps be applied and they may lead to the preparation of an effective vaccine against *H. capsulatum*.

(2) The presence of resistance-influencing endotoxin like substances is probable in *H. capsulatum*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Blastomyces dermatitidis* and *Sporotrichum Schenckii*. Methods similar to those used in this paper for *Candida* may perhaps be utilized to confirm their presence in these fungi and examine their effect on nonspecific immunity.

(3) An investigation of the presence and localization of the specific antigens and of sensitizing substances in these and other fungi may be fruitful steps toward devising effective protective antifungal vaccines. A definitive identification of the various immunologically active fractions of *Candida* on electron microscopy and a comparative study of other fungi, with a search for corresponding anatomical structures may besides biochemical methods, lead to this objective.

(1) *Possible Application of the Reported Findings in Therapy and Prevention of Systemic Mycoses*

The knowledge of the function of some cell constituents of *Candida* reported here, and the possibility that similar mechanisms may be operative in other systemic mycoses, may help influence therapeutically by immunologic means, some of the specific and nonspecific mechanisms in systemic mycoses. This may result in a more successful treatment of these diseases. Some possible therapeutic measures will be discussed here.

(1) del Pont (16) Hoffmeister Dickenger and Göting (48) Connor (15) Fomina and Stepanishcheva (34) and Ata (1) have shown that specific immunity to *Candida* may be passively transferred. A therapeutic effect in systemic mycoses may therefore, be expected from a *passive transfer of specific antibodies* by means of injections of serum from animals which had been immunized against the fungus, preferably by the use of cell walls or some of their chemical fractions, to eliminate the possible passive transfer of hypersensitivity which was demonstrated by Kesten and Mott (61).

(2) It has been realized that not much benefit could be expected from *active therapy* in rapidly developing acute infections. Since, however most systemic fungus infections, including moniliasis, are usually decidedly chronic conditions, there would be time and probably need for longer lasting specific protection by active immunization. The experiments of Concetti (14) and Fomina and Stepanishcheva (34) dem-

onstrate the feasibility of this approach. It would appear important, especially for this purpose, to use vaccines devoid of the endotoxin-like substance so that the growth of *Candida* already present in the human or animal organism is not enhanced. It would also be important to use vaccines from which allergenic products have been removed. Vaccine therapy with a cell wall preparation, if possible one containing a single antigenic component such as a cell wall polysaccharide, would, therefore, appear to be indicated as an additional therapeutic measure in systemic fungus infections.

(3) The use of measures directed toward inactivation of the endotoxin-like substance of *Candida* which apparently stimulates constantly the growth of the fungus in cases of extensive moniliasis (pages 61-62) has been discussed on pages 60-61. The use of these measures may apply to other systemic fungus infections as well. The importance of an attempt to inactivate bacterial endotoxin in systemic moniliasis enhanced by the use of antibiotics would appear obvious in the light of the discussion on pages 58-61.

(4) In view of the finding of several authors that hypersensitivity is one of the possible mechanisms for the establishment of disseminated moniliasis *desensitization* may be indicated as a useful adjunct to therapy at least in some cases of systemic mycoses (82). It is possible that the cases of Grossi and Balog (39) and the patient of Erdm and Finlayson (25) may have improved as a result of desensitization with heat killed whole cells of *Candida*.

(5) A general use of *preventive vac-*

nation against fungus diseases does not seem indicated because the exposure and susceptibility to the various fungus diseases appear to be limited to certain age groups, occupations and geographic areas. Epidemics of moniliasis do not seem to be common but they do occur especially in young infants institutionalized for long periods of time, where a danger of cross-infection with moniliasis, which may be at times fatal is high (14 26 103 142) Under special circumstances under which the danger of exposure susceptibility and occurrence of epidemics of fungus diseases is high immunization may prove to be very useful and possibly lifesaving Immunization against histoplasmosis, coccidioidomycosis and other systemic mycoses of limited geographic distribution would apparently be useful, at least for certain segments of the population. It has been stated that the problem of coccidioidomycosis would be essentially solved by effective vaccination of susceptible persons (27) For reasons stated before cell wall vaccines should be used for this purpose providing the same general principles concerning the localization of the protective antigen, the existence and localization of endotoxin-like substances and of materials responsible for sensitization applying to *Candida* apply also to the causative agents of other systemic mycoses (page 64)

(3) *Possible Bearing of the Reported Findings on the Pathogenesis and Management of Bacterial Infections Arising During Antibiotic Treatment*

The tentative explanation for the enhancement of *Candida* infections during antibiotic therapy may have a wider application beyond the field of fungus diseases. It is conceivable that the growth of organisms other than *Candida* (*Pseudomonas Aerobacter Proteus Staphylococcus*) which are often not susceptible to the action of the commonly used antibiotics, may be stimulated by the endotoxins liberated from the intestinal flora which is destroyed by the action of antibiotics.

This may answer the question why these organisms which had only rarely been the cause of generalized fatal disease before the era of antibacterial drugs have been becoming increasingly dangerous since. The statistical data given by Finland Jones and Barnes (29) illustrate impressively the magnitude of this problem.

The development of adequate measures to counteract the effects of endotoxin, possibly on the basis of the work summarized on pages 60-61 may decrease the effects of this enhancement in numbers, pathogenicity and invasiveness of bacterial organisms which are not susceptible to antibiotics in patients subjected to antibiotic treatment.

## (5) SUMMARY

(1) Our experiments have shown that a significant number of mice repeatedly injected subcutaneously with washed cell walls of *Candida albicans* were protected against intravenous challenge with viable cells of the same fungus which followed 21 days after the last injection. In a similar experimental setting no protection resulted from repeated injections of soluble extract of *C. albicans* prepared by mechanical disintegration of its yeast cells.

(2) In a series of experiments mice were given one injection of either the endotoxin of *Escherichia coli* or the soluble extract of *C. albicans* followed 5, 8, 14 and 30 days later by an intravenous challenge with *C. albicans*. The survival time of mice challenged 5, 8 or 14 days after the pretreatment was prolonged. The survival times of mice were shortened when either *E. coli* endotoxin or the soluble extract of *C. albicans* were injected after the *Candida* infection.

Similar results were obtained when the challenge with *Candida* was substituted by an intraperitoneal challenge with *Salmonella enteritidis*.

The effect of the endotoxin or the *Candida* extract was much more pronounced on intraperitoneal than on subcutaneous injection. The activity of the *Candida* extract was preserved after heating.

(3) Anaphylactic shock was produced

in the majority of guinea pigs sensitized with the soluble extract of *Candida* but not with the cell walls.

(4) An extensive review of past experimental work on nonspecific host resistance, hypersensitivity and its relation to immunity and on specific immunity in moniliasis is given.

(A)(i)(a) Data in the literature indicate that *Candida* infections may be enhanced by subsequent treatment with dead gram-negative bacterial organisms or their endotoxins, or by dead cells of *Saccharomyces cerevisiae*. A partial protection against a *Candida* infection may be achieved by pretreatment with endotoxins of *Salmonella enteritidis* or *S. typhosa*, with nonviable spherules of *Coccidioides immitis*, sterile milk or Freund's adjuvant.

(A)(i)(b) It was shown that repeated injections of dead cells of *C. albicans* given after infection with the same fungus enhance its growth while dead cells given several days before challenge have a protective effect.

(A)(i)(c) Other experiments show that dead *Candida* or its extracts given simultaneously with or shortly before a challenge with *Salmonella typhosa*, *Staphylococcus Potens*, *Pseudomonas* or *Mycobacterium tuberculosis* enhance the bacterial infections.

(A)(ii)(a) Living *Proteus Pseudomonas* or *Saccharomyces* but not *St. pio-*

*coccus* *Staphylococcus* or *Bacillus subtilis* injected simultaneously with or shortly after *Candida* caused much higher mortalities in mice than injections of single organisms. *Escherichia coli* injected simultaneously with *C. albicans* caused only a slight increase in death rates a phenomenon possibly due to the presence in *E. coli* of an antibiotic substance effective against *Candida*. A prolongation of life was noted in mice injected with *Histoplasma capsulatum* *Cryptococcus neoformans* *Coccidioides immitis* or *Aspergillus fumigatus* 6 days before challenge with *C. albicans*. Simultaneous infections with *C. albicans* and *Mycobacterium tuberculosis* caused enhancement of the tuberculous infection. This may be due to the effects taking place in the previously mentioned experiments, viz. the presence of resistance-modifying fractions in the bacterial and fungal organisms, and to an in vitro active growth promoting polysaccharidic extract from *C. albicans*.

(A)(1)(b) Small intraperitoneal injections of viable cells of *C. albicans* repeated every 2-4 days killed more animals than a single large intraperitoneal injection containing a number of organisms larger than that administered in all small injections combined. When the interval between 2 large intraperitoneal injections of *C. albicans* was 10 days more animals died than when the interval was 90 days. The time of death of animals intravenously infected with *Candida* was delayed when they were pretreated with the same organism by intraperitoneal route 6 days earlier.

(B) Attempts to produce broncho-

pulmonary moniliasis by intratracheal inoculation of *Candida* were successful in animals sensitized with heat killed cells of *Candida* but not in non-sensitized animals. The authors of these experiments concluded that sensitization was one of the possible mechanisms for dissemination of moniliasis. It was also possible to sensitize rabbits passively to *Candida*.

(C) Most experiments with the aim to confer immunity to *Candida* have been unsuccessful.

(1) The reason for the lack of success may have been allergic sensitization or the presence of the endotoxin like substance of *Candida* in the immunizing injections and its adverse effect on non-specific resistance. When viable organisms are used for immunization the endotoxin-like substance may enhance the growth of the fungus contained in each subsequent vaccine dose or in the challenge dose. In animals immunized by intravenous or intraperitoneal route this effect is more pronounced. The administration of the vaccines by these routes has another disadvantage, viz. that viable organisms in the vaccines may cause generalized moniliasis more easily.

(11) An analysis of the few successful immunization experiments reveals that dead cells were used in almost all of them at least initially. This eliminated the constant liberation of the endotoxin-like substance. Early availability of the specific antigen may have explained the success of vaccination experiments with cell walls and with sonically vibrated and acid treated cells. Sonic vibration may have caused easier availability of the protective cell wall antigen which

may be located in an inside layer of the cell wall. Treatment with sulfuric acid may have dissolved the outer mannan-protein layer and exposed the inner layer of glucan-protein which may contain the protective antigen. In some experiments the vaccine injections were timed in such a manner that it may be speculated that increase in nonspecific resistance produced by the endotoxin-like substance may have potentiated the effect of the specific protective antigen. In almost all successful experiments injections were given subcutaneously. This decreased the danger of generalization of the infection which may take place when living cells are used for immunization. The danger of sensitization may have been decreased in some experiments by the use of formalin-killed cells, in others by the treatment of yeast cells with sulfuric acid, in still others perhaps by desensitization achieved by a gradual increase of dosage and change of route of administration from subcutaneous to intravenous.

(iii) Vaccine therapy of animals already infected with *Candida* as well as passive immunization were reported to be successful. In some instances the lack of effect of passive immunization may have been due to passive transfer of hypersensitivity. Serum therapy of infected animals is also possible.

(5) The soluble fraction of *C. albidus* described in the experimental part apparently contains substances from the cytoplasm, possibly also from the capsule covering the outer surface of the cell wall and perhaps some soluble constituents of the cell wall. It produces sensitization and contains the endotoxin-

like substance responsible for the modification of nonspecific host resistance. Protection against homologous challenge may be produced by the cell wall preparation which, however also causes a mild local tissue irritation. An ideal protective vaccine should contain a single antigenic component to eliminate side effects.

A critical analysis of experimental findings of others points to the possibility that the protective antigen of *C. albicans* may be located in the inside layer of the cell wall. Chemical and electron microscopic studies reveal that the outer layer of the cell wall of *C. albicans* consists of a glucomannan-protein complex while the inner layer is made up of a glucan-protein complex containing less protein than the outer layer.

(6) The administration of gram-negative endotoxins, pertussis vaccine killed *Mycobacterium tuberculosis* symptom, mucin, colloidal sulfur silica dust, sterile milk and other substances has been found to influence host resistance to gram-negative organisms, mycobacteria, staphylococci and some viruses. An injection of one of these substances is followed by several hours to several days of decreased resistance after which resistance is increased for several days to several weeks. Injections of these substances given simultaneously with or after bacterial infections shortened the life of infected animals. Chronic bacterial infections may thus be converted into acute ones.

(7) The substance present in the soluble extract of *C. albicans* which is apparently responsible for similar changes in nonspecific host resistance is referred

to as "endotoxin-like" since it has toxic and hematologic properties similar to those of gram-negative endotoxins.

(8) (a) Destruction, during antibiotic treatment, of large numbers of organisms of the normal gastrointestinal flora containing also gram negative endotoxin producing bacteria may cause sudden liberation of large amounts of endotoxin. This may similarly as an injection of endotoxin in own experiments, enhance the growth of microorganisms which are present in the host and are susceptible to the resistance influencing action of endotoxins but which are resistant to the inhibitory action of the administered antibiotic. Among them *Candida* is one of the common inhabitants of the gastrointestinal tract.

(b) The increased incidence of severe infections due to bacterial organisms such as *Pseudomonas* *Aerobacter* *Proteus* and *Staphylococcus* which used to cause such diseases only rarely before the introduction of antibiotics, may possibly be explained on the same basis.

(c) Cases of disseminated moniliasis following immunization with pertussis vaccine or BCG vaccination may be due to the action of the experimentally demonstrated resistance-influencing properties of these vaccines.

(d) One of the reasons for the multiplication of *Candida* in systemic moniliasis may be the constant liberation of the endotoxin like substance from large numbers of disintegrating *Candida* organisms.

(e) If these speculations are correct it may be worth while to try to counteract the spread of the infections enhanced by bacterial endotoxins and other re-

sistance modifying substances by the use of measures directed toward the inactivation of endotoxins, e. g. the use of serum factors active against endotoxins. Since the chemical characteristics of the resistance influencing substances other than bacterial endotoxins are unknown one may not speculate whether or not they may be inactivated by the same methods.

(9) On the basis of own experiments and of the data from the literature one may conclude that the following immunological measures may be helpful in the management of systemic moniliasis.

(a) Therapy with a cell wall vaccine may be indicated for conferring a specific immunity. To eliminate infection-enhancing, allergic and local side effects such a vaccine should preferably contain a single protective antigenic substance which may be located in the inner layer of the cell wall. It should not include any fractions present in the soluble extract which contains a sensitizing and an endotoxin like substance.

(b) Treatment with serum of animals immunized with a cell wall preparation may be more promptly effective and may be used as an adjunct to the active vaccine therapy. The use of cell walls only for the immunization of animals may eliminate passive transfer of hypersensitivity to patients.

(c) Measures directed toward inactivation of the endotoxin-like substance of *Candida* as well as of bacterial endotoxins which may stimulate the growth of *Candida* may be helpful in patients with systemic moniliasis. These measures may or may not be identical, and

may include the use of endotoxin-inactivating serum factors.

(d) Desensitization may be a useful part of therapy since it was shown that hypersensitivity is one of the possible mechanisms for the generalization of moniliasis.

( ) A preventive use of vaccination with a cell wall preparation may be useful in certain age groups or in persons heavily exposed to the fungus.

(10)(a) The presence of resistance-influencing endotoxin-like substances is probable in *Histoplasma capsulatum*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Blastomyces dermatitidis*, *Sporotrichum Schenckii* and in members of the genus *Candida* other than *albicans*.

(b) It may be concluded that *Histoplasma capsulatum* has a close immunological similarity to *C. albicans*. It appears to possess, like *Candida*, not only an endotoxin-like substance, but also a protective antigen located in the cell wall and a sensitizing substance in its cytoplasm. An effective vaccine against this fungus may perhaps be prepared on the basis of the principles described here

for *Candida* by using a cell wall antigen and eliminating any fractions present in its soluble extract which probably contains a sensitizing and a resistance-influencing endotoxin-like substance.

(c) An identification of the various immunologically active fractions of *Candida* on electron microscopy and a comparative electron microscopic study of other fungi, with a search for corresponding anatomical structures may besides biochemical methods, indicate whether the preparation of similar anti-fungal vaccines may be feasible using such fungi.

(d) Immunological measures similar to those suggested under (9) (a) to (e) for the management of systemic moniliasis may be useful in the fungus diseases the causative agents of which would reveal to have essential immunological features analogous to those of *Candida*. An effective preventive vaccine against histoplasmosis, coccidioidomycosis and other systemic mycoses of limited geographic distribution may solve many public health problems posed by these diseases.

## ACKNOWLEDGEMENT

This work was supported by Research Grant CA-03267-07 and by the General Research Support Grant from the National Institutes

of Health of the United States Public Health Service.



to as "endotoxin-like" since it has toxic and hematologic properties similar to those of gram-negative endotoxins.

(8)(a) Destruction, during antibiotic treatment, of large numbers of organisms of the normal gastrointestinal flora containing also gram-negative endotoxin-producing bacteria may cause sudden liberation of large amounts of endotoxin. This may similarly as an injection of endotoxin in own experiments, enhance the growth of microorganisms which are present in the host and are susceptible to the resistance-influencing action of endotoxins but which are resistant to the inhibitory action of the administered antibiotic. Among them *Candida* is one of the common inhabitants of the gastrointestinal tract.

(b) The increased incidence of severe infections due to bacterial organisms such as *Pseudomonas*, *Aerobacter*, *Proteus* and *Staphylococcus* which used to cause such diseases only rarely before the introduction of antibiotics, may possibly be explained on the same basis.

(c) Cases of disseminated moniliasis following immunization with pertussis vaccine or BCG vaccination may be due to the action of the experimentally demonstrated resistance-influencing properties of these vaccines.

(d) One of the reasons for the multiplication of *Candida* in systemic moniliasis may be the constant liberation of the endotoxin-like substance from large numbers of disintegrating *Candida* organisms.

(e) If these speculations are correct it may be worth while to try to counteract the spread of the infections enhanced by bacterial endotoxins and other re-

sistance-modifying substances by the use of measures directed toward the inactivation of endotoxins, e. g. the use of serum factors active against endotoxins. Since the chemical characteristics of the resistance-influencing substances other than bacterial endotoxins are unknown one may not speculate whether or not they may be inactivated by the same methods.

(9) On the basis of own experiments and of the data from the literature one may conclude that the following immunological measures may be helpful in the management of systemic moniliasis.

(a) Therapy with a cell wall vaccine may be indicated for conferring a specific immunity. To eliminate infection-enhancing allergic and local side effects such a vaccine should preferably contain a single protective antigenic substance which may be located in the inner layer of the cell wall. It should not include any fractions present in the soluble extract which contains a sensitizing and an endotoxin-like substance.

(b) Treatment with serum of animals immunized with a cell wall preparation may be more promptly effective and may be used as an adjunct to the active vaccine therapy. The use of cell walls only for the immunization of animals may eliminate passive transfer of hypersensitivity to patients.

(c) Measures directed toward inactivation of the endotoxin-like substance of *Candida* as well as of bacterial endotoxins which may stimulate the growth of *Candida* may be helpful in patients with systemic moniliasis. These measures may or may not be identical, and

- injection of pertussis vaccine or of bacterial endotoxin. *J. Exptl. Med.* 104 53—63.
0. Dubos, R. J. and R. W. Schaedler 1957 Effects of cellular constituents of mycobacteria on the resistance of mice to heterologous infections. I. Protective effects. *J. Exptl. Med.* 106 703—717.
  1. Dubos, R. J. and R. W. Schaedler. 1960 The effect of the intestinal flora on the growth rate of mice, and on their susceptibility to experimental infections. *J. Exptl. Med.* 111 407—417.
  2. Dubos, R. J. and R. W. Schaedler 1962 The effect of diet on the fecal bacterial flora of mice and on their resistance to infection. *J. Exptl. Med.* 115 1161—1172.
  3. Eddy A. A. 1958 The structure of the yeast cell wall. II Degradative studies with enzymes. *Proc. Roy. Soc. (London) B.* 149 425—440.
  4. Elisev N. P. and V. V. Bystron. 1961 The cause of the possible death of rabbits on immunization with yeasts. *J. Microbiol. Epidemiol. Immunobiol. (U.S.S.R.)* 32 82—87.
  5. Erdos, W. and M. H. Finlayson. 1934 Meningitis of the central nervous system in child with recovery. *B. African Med. J.* 28 868—871.
  6. Faber H. K. and E. B. Clark. 1927 Prevention and treatment of thrush (oral stomatitis). *Am. J. Diseases Children* 34 408—417.
  7. Flew M. J. 1938 *Coccidioidomycosis*. Charles C. Thomas, Springfield, Illinois. Pp. 174—175.
  8. Fine, J. S. Rutensburg, and F. B. Schwensburg 1959 The role of the reticulo-endothelial system in hemorrhagic shock. *J. Exptl. Med.* 110 547—569.
  9. Finland, M. W. F. Jones, J. and M. W. Barnes 1959 Changes in the occurrence of serious bacterial infections since the introduction of antibacterial agents. *Trans. Assoc. Am. Physicians* 72 303—372.
  30. Fischer M. W. 1955 Die Soorkomplikation der Aureomycintherapie im Lichte therapeutischer Untersuchungen. *Ann. Univ. Saravienae. Serie Medicina* 3 103—166.
  31. Fischer G. W. and L. Horbach. 1958 Untersuchungen über Promucil und Infektionssuszeptibilität bei der experimentellen Soorkinfektion. *Arch. Hyg. u. Bakteriol.* 142 14—25.
  32. Fischer J. 1959 Zur Beeinflussung der Serumkaktoidose. Ein Beitrag zur Frage der spezifischen Resistenz. *Schweiz. Z. allgem. Pathol. u. Bakteriologie* 22 827—843.
  33. Flachl, R. 1919 Entwicklung und gegenwärtiger Stand unserer Kenntnisse über die Soorkrankheit. *Ergeb. inn. Med. u. Kinderheilk.* 16 107—191.
  34. Fomina, I. P. and Z. G. Stepanishcheva. 1957 Model' eksperimental'nogo ostrogo vnutrannego kandidomikozn myshel es ispol'zovanie dlia isshetna terapevticheskoi effektivnosti protivodrozdobnykh antibioticheskikh i khamioterapevticheskikh preparatov (An experimental model of an acute visceral moniliasis of mice and its use for the investigation of therapeutic effects of antibiotics and chemotherapeutic agents against yeasts.) *Antibiotiki* 2 34—38.
  35. Freyckman, S. K. L., S. O. Peterson, and B. Steenberg. 1955, Coliformin production and isolation. *Antibiotics & Chemotherapy* 5 218—223.
  36. Gale, D. and M. Devesty 1957 Response of mice to inoculations of both *Candida albicans* and *Escherichia coli*. VI. The effect of the status of mice. *J. Infectious Diseases* 101 48—50.
  37. Gale D. and B. Sandkov. 1957 Response of mice to the inoculations of both *Candida Albicans* and *Escherichia coli*. I The enhancement phenomenon. *J. Bacteriol.* 73 616—644.

## (6) LITERATURE CITED

- 1 Ata, S. 1957 Kreuzungsreaktion bei der Hämagglutination zwischen *Candida Albicans* und *Mycobacterium Tuberculosis*. Zentr. Bakteriell. Parasitenk. Abt. I Orig. 170 100—103
- 2 Banič, S. 1960 Antagonistische Wirkung von Enterobacteriaceen auf Staphylokokken und *Candida albicans*. Zentr. Bakteriell. Parasitenk. Abt. I Orig. 180 27—29
- 3 Benacerraf, B., M. M. Sebestyen, and S. Schlossman. 1959 A quantitative study of the kinetics of blood clearance of <sup>125</sup>I-labelled *Escherichia coli* and staphylococci by the reticuloendothelial system. J. Exptl. Med. 110 27—48.
- 4 Bensoussan Cherboul, L. 1955 Contribution à l'étude du traitement des cas graves d'infection à *Candida albicans* par la Fungicide. Medical thesis No 931 Paris
- 5 Bishop, C. T., F. Blank and P. Gardner. 1960 The cell wall polysaccharides of *Candida albicans*: glucan, mannan, and chitin. Can. J. Chem. 38 869—881
- 6 Bohme H. 1958. Mechanismen der unspezifischen Infektionsresistenz. Klin. Wochschr. 36 837—845
- 7 Bohme D. and C. A. Bourvier. 1960 Über den Einfluss bakterieller Fraktionen auf Gewebe, reticuloendotheliales System und Differentialblutbild der Albinos. Beitr. path. Anat. u. allgem. Pathol. 122 188—198.
- 8 Brandis, H. 1954 Über die Promilität (Depressionsimmunität). Ergeb. Hyg. Bakteriol. Immunitat. rech. u. Exptl. Therap. 28 141—202
- 9 Braude A. I. and J. Siemieniuk. 1961 The influence of endotoxin on resistance to infection. Bull. N. Y. Acad. Med. (2) 37 448—467
- 10 Cavallero C. 1937 Observations sur la biologie du « champignon du muguet » [*Mycotorula albicans* (Roben) Langeron et Talice 1932] Note IV. Immunité active acquise par *Mycotorula albicans*. Expériences de vaccination et de préinoculation. Boll. sez. Ital. soc. Intern. microbiol. 9 262—267
- 11 Charrin and Ostrowsky. 1896. *Lodinium albicans*, agent pathogène général. Compt. rend. soc. biol. 48 743—744.
- 12 Cohn Z. A., and S. I. Morse. 1960 Functional and metabolic properties of polymorphonuclear leucocytes. II. Influence of a lipopolysaccharide endotoxin. J. Exptl. Med. 111 689—704
- 13 Conant N. F., D. T. Smith, R. D. Baker, J. L. Callaway and D. S. Martin. 1954. Manual of Clinical Mycology. Ed. 2. W. B. Saunders Company Philadelphia.
- 14 Concetti L. 1900 Biologie et pathogénie du muguet. Arch. méd. enfants 3 449—479 517—541 590—605
- 15 Connor C. L. 1928. Monilia from osteomyelitis. J. Infectious Diseases 43 108—116
- 16 del Pont, A. M. J. 1904 Estudio sobre la inmunidad del muguet. Anales dir. cul. méd. arg. 27 331—340.
- 17 Dobson, R. 1957 Moniliasis in pediatrics. A. M. A. J. Diseases Children 94 234—231
- 18 Donnan, J. and Y. Kawamor. 1955 Moniliasis. J. Antibiotics (Japan) Ser. A. 8 171—180
- 19 Dubos, R. J. and R. W. Schaedler. 1956 Reversible changes in the susceptibility of mice to bacterial infections. I. Changes brought about by

- injection of pertussis vaccine or of bacterial endotoxin. *J. Exptl. Med.* 104 53—63
20. Dobos, R. J. and R. W. Schaedler 1957 Effects of cellular constituents of mycobacteria on the resistance of mice to heterologous infections. I. Protective effects. *J. Exptl. Med.* 106 703—717
  21. Dobos, R. J. and R. W. Schaedler 1960 The effect of the intestinal flora on the growth rate of mice, and on their susceptibility to experimental infections. *J. Exptl. Med.* 111 407—417
  22. Dobos, R. J. and R. W. Schaedler 1962 The effect of diet on the fecal bacterial flora of mice and on their resistance to infection. *J. Exptl. Med.* 115 1161—1172
  23. Eddy, A. A. 1958. The structure of the yeast cell wall. II. Degradative studies with enzymes. *Proc. Roy. Soc. (London) B.* 149 425—440.
  24. Elinov, N. F. and V. V. Dystrova 1961 The cause of the possible death of rabbits on immunization with yeasts. *J. Mikrobiol. Epidemiol. Immunobiol. (U.S.S.R.)* 32 82—87
  25. Ewold, W. and M. H. Finlayson. 1954 Moulds of the central nervous system in child with recovery. *S. African Med. J.* 58 868—871
  26. Faber, H. K., and E. B. Clark 1957 Prevention and treatment of thrush (oral moniliasis). *Am. J. Diseases Children* 34 406—417
  27. Fleiss, M. J. 1956. *Coccidioidomycosis*. Charles C. Thomas, Springfield, Illinois. Pages 174—175
  28. Fine, J. S., Rutenburg, and F. B. Schwennberg 1959 The role of the reticulo-endothelial system in hemorrhagic shock. *J. Exptl. Med.* 110 547—569
  29. Finland, M., W. F. Jones, J. and M. W. Barnes 1959 Changes in the occurrence of serious bacterial infections since the introduction of anti-bacterial agents. *Trans. Assoc. Am. Physicians* 72 305—322.
  30. Flecher, G. W. 1955. Die Soorkomplika-tion der Aureomycintherapie im Lichte tierexperimenteller Untersuchungen. *Ann. Univ. Karlovienae. Serie Me-dizin* 3 105—266.
  31. Flecher, G. W. and L. Horbach. 1958. Untersuchungen über Promunität und Infektionsimmunität bei der experi-mentellen Soorinfektion. *Arch. Hyg. u. Bakteriol.* 142 14—25
  32. Flecher, J. 1959 Zur Beobachtung der Soorbakterioidie. Ein Beitrag zu Frage der unspezifischen Resistenz. *Schweiz. Z. allgem. Pathol. u. Bakte-riol.* 22 827—845
  33. Flechl, R. 1918. Entwicklung und gegenwärtiger Stand unserer Kenntnisse über die Soorkrankheit. *Ergeb. inn. Med. u. Kinderheilk.* 18 107—191
  34. Fonina, I. P. and Z. G. Stepanichyeva. 1957 Model' eksperimental'nogo ostrogo vnutrannego kandidomikozn trybel' na kopol'sovaniye dlya izochet-nay respektivnoykh effektivnoy protivodrozhdevykh antisepticheskikh i khimioterapevticheskikh preparatov (An experimental model of an acute visceral moniliasis of mice and its use for the investigation of therapeutic effects of antibiotics and chemotherapeutic agents against yeasts). *Antibio-tiki* 2 34—38
  35. Freyckunst, S. K. L. S. O. Pehrson, and B. Stenberg. 1955 *Cofformin* pro-duction and isolation. *Antibiotics & Chemotherapy* 5 218—223
  36. Gale, D. and G. Dersuty 1957 Re-sponse of mice to inoculations of both *Candida albicans* and *Escher-ichia coli*. II The effect of the strain of mice. *J. Infectious Diseases* 101 48—50
  37. Gale, D. and B. Sandoval 1957 Re-sponse of mice to the inoculations of both *Candida albicans* and *Escher-ichia coli*. I. The enhancement phe-nomenon. *J. Bacteriol.* 73 616—624

- 37a. Gale G R. 1963 Cytology of *Candida albicans* as influenced by drugs acting on the cytoplasmic membrane. *J Bacteriol.* 86 151—157
38. Gausewitz P L., F S. Jones, and G Worley Jr. 1951 Fatal generalized moniliasis. Report of a case. *Am. J Clin. Pathol.* 21: 41—49
39. Gross, G. and P. Balog. 1959 Clinical and experimental studies in *Castellan's* pulmonary moniliasis. *J Trop Med. Hyg.* 32 253—262
40. Hasenclever H. F., and W. O. Mitchell. 1962. Production in mice of tolerance to the toxic manifestations of *Candida albicans*. *J Bacteriol.* 84 402—409
41. Hasenclever H. F. and W. O. Mitchell. 1962. Production of tolerance to the toxicity of *Candida albicans* by non-fungal materials. *J Bacteriol.* 84 1325—1329
42. Hedgecock, L. W. 1962. Effect of vaccines prepared from *Histoplasma capsulatum* and other yeasts on experimental tuberculosis. *J Bacteriol.* 82 115—123
43. Hegemann F. 1957 Studien über die Natur des Sickerstoffneutralisierenden Faktors im normalen menschlichen Blut. IV Mitteilung. Der Nachweis der endotoxinneutralisierenden Eigenschaft des menschlichen Blutes durch den Tierversuch. *Z. Immunitätsforsch.* 114 1—9
44. Henrici, A. T. 1940 Characteristics of fungous diseases. *J Bacteriol.* 39 113—138.
45. Hiatt, J. S. and D. S. Martin. 1946 Recovery from pulmonary moniliasis following serum therapy. *J Am. Med. Assoc.* 130 205—206
46. Hill G. A. 1958 Host resistance in systemic mycotic infections. Ph.D. Thesis. Univ. of Utah (L.C. Card No. Mic 58—7919) 98 p. Univ. Microfilms. Ann Arbor Mich.
47. H. M., and E. H. Kass. 1957 A plasma factor that protects against the lethal action of end toxin. *J Clin. Invest.* 36 900
48. Höffmeyer W. F. Dickpfeiser and H. Göting. 1951 Tierexperimentelle und serologische Untersuchungen zur Diagnostik und Therapie der Infektion mit *Candida albicans*. *Deut. Arch. klin. Med.* 198 499—508.
49. Hook, W. A., and L. H. Muschel. 1959 Lysozyme and properdin levels in mice treated with zymosan endotoxin or x irradiation. *Federation Proc.* 18 573.
50. Höder M. H., B. Kickhöfen, and F. Wendt. 1958. Aktivierung der Fibrinolyse beim Menschen durch ein bakterielles Pyrogen. Der Einfluss von Phenylbutazon und Heparin auf Fibrinolyse, Blutgerinnung und Fieberreaktion. *Klin. Wochsch.* 36 164—166.
51. Hourwink, A. L., and D. R. Kregler. 1953 Observations on the cell wall of yeasts. An electron microscope and x ray diffraction study. *Antonie van Leeuwenhoek. J. Microbiol. Serol.* 19 1—4
52. Howard, J. G. D. Rowley and A. C. Wardlaw. 1958 Investigations on the mechanism of stimulation of non-specific immunity by bacterial lipopolysaccharides. *Immunology* 1 181—203
53. Hurd R. C. and C. H. Drake. 1955. *Candida albicans* infections in actively and passively immunized animals. *Mycopathol et Mycol. Appl.* 6 290—297
54. Isenberg H. D. J. Allerhand, and J. I. Berkman. 1963 An endotoxin-like fraction extracted from the cells of *Candida albicans*. *Nature (London)* 197 516—51
55. Janoff A. and B. W. Zweisach. 1960 Inactivation of bacterial exotoxins and endotoxin by iron. *J Exptl. Med.* 112 73—84
56. Janssen, H. and D. Boldé. 1930. Les lésions Clavovaccina méliorata. Technique de préparation. *Compt. rend. soc. biol.* 104 455—456.
57. Janssen, H., and R. Schier. 1930. Les clavo-vaccins. Vaccino-thérapie des dermatomycoses et de leurs séquelles allergiques. *Presse méd.* 38 621—625

58. Kefler, W. 1936. *Sporangium* nach Impfung KfG. *Wochsche. 15* 683—684.
59. Keppie, J. E. C. Cocking and H. Smith. 1938. A non-toxic complex from *Pasteurella pestis* which immunizes both guinea-pigs and mice. *Lancet* 1 246—247.
60. Kewler G. and W. J. Kirkerson. 1939. Glucosaminase-protein complexes from cell walls of yeasts. *J. Biol. Chem.* 234 2281—2285.
61. Kesten, H. H. and E. Mott. 1931. Hypersensitiveness to soluble specific substances from yeast-like fungi. I. *Anaphylaxis*. *J. Exptl. Med.* 53 803—814.
62. Kier J. S. H. Lindh, and G. G. de Mello. 1956. The effect of various substances on resistance to experimental infections. *Ann. N.Y. Acad. Sci.* 65 312—328.
63. Klippman, A. M. and E. D. DeLamater. 1950. The immunology of the human mycoses. *Ann. Rev. Microbiol.* 4 283—312.
64. Krodolfer F. and R. Wyler. 1938. Unspezifische Resistenzfaktoren bei Virusinfektionen. *Schweiz. Z. allgem. Pathol. u. Bakteriologie* 21 83—88.
65. Krodolfer F. R. Wyler and R. Meier. 1937. Abhängigkeit der Antikörperbildung bakterieller Polysaccharide von Behandlungsernährung, Behandlungsdosis und Infektionsart. *Experientia* 13 187—189.
66. Kurochkin, T. J. and C. E. Lam. 1930. Anaphylaxis with water soluble specific substance from yeastlike fungi. *Proc. Soc. Exptl. Biol. Med.* 28 223—225.
67. Kurochkin, T. J. and C. E. Lam. 1931. Effect of formaldehyde upon the sensitizing property of Monilia. *Proc. Soc. Exptl. Biol. Med.* 29 237—239.
68. Kurochkin, T. J. and C. E. Lam. 1933. Experimental bronchomonilliasis in anesthetized rabbits. *Proc. Soc. Exptl. Biol. Med.* 31 332—334.
69. Landy M. 1936. Increase in resistance following administration of bacterial lipopolysaccharides. *Ann. N.Y. Acad. Sci.* 65 292—303.
70. Landy M., and L. Pillemey. 1936. Elevation of properdin in mice following administration of bacterial lipopolysaccharides. *J. Exptl. Med.* 163 823—833.
71. Landy M., R. C. Skarnes, F. S. Rosen, R. J. Trapani, and M. J. Skea. 1937. Inactivation of biologically active (endotoxic) polysaccharides by fresh human serum. *Proc. Soc. Exptl. Biol. Med.* 96 744—747.
72. Lederer H. and R. McL. Todd. 1949. Thrush in infancy. *Arch. Disease Childhood* 24 200—207.
73. Levine H. B. 1962. Immunogenicity of experimental vaccines in systemic mycoses, p. 254—276. / G. Dalldorf (ed.) *Fungi and fungous diseases. A symposium of the Section of Microbiology The New York Academy of Medicine.* Charles C. Thomas, Springfield, Illinois.
74. Lam, C. E. and T. J. Kurochkin. 1932. Actin bacterial anaphylaxis in relation to antibody production. *Proc. Soc. Exptl. Biol. Med.* 29 1151—1153.
75. Lam, C. E. and T. J. Kurochkin. 1936. The length of incubation time in actin anaphylaxis produced with cultures of *Monilia*. *Chinese Med. J. Suppl.* 1 Feb. 1936 256—260.
76. Lam, C. E. T. J. Kurochkin, and C. J. W. 1930. Experimental bronchomonilliasis in rabbits. *Nat. Med. J. China* 16 537—544.
77. Louria, D. B. 1960. Specific and non-specific immunity in experimental cryptococcosis in mice. *J. Exptl. Med.* 111 643—653.
78. Mankiewicz, E. and M. Lusak. 1960. Effect of *Candida albicans* on the evolution of experimental tuberculosis. *Nature (London)* 187 250—251.
79. Mankiewicz, E., E. Stachewicz, and M. Lusak. 1959. A polysaccharide isolated from *Candida albicans* as growth-promoting factor for *Mycobac*

- terium tuberculosis. Can. J. Microbiol. 5 261—267
- 80 Marcus, S. and F. R. Rambo. 1955 Comparative aspects of immunization of mice against systemic mycoses. Bacteriol. Proc. Soc. Am. Bacteriologists 55 92.
- 81 Markenson, J. B. Sultzeanu and A. L. Olitzki. 1967 Immunogenic activity of Brucella cell wall. Brit. J. Exptl. Pathol. 43 67—76
- 82 Martin D. S. 1941 The practical application of some immunologic principles to the diagnosis and treatment of certain fungus infections. J. Invest. Dermatol. 4 471—481
- 83 Mazzetti, G. and G. Fiesi Marracchini. 1957 Primi dati sperimentali su una vaccinoprophilassi e una vaccinoterapia dell'infezione da *Candida albicans*. Giorn. mal. infettive e parassit. 9 323—325
- 84 Meier R., H. J. Bein, and R. Jacques. 1957 The action of bacterial polysaccharides on allergic phenomena. Intern. Arch. Allergy Appl. Immunol. 11 101—118.
- 85 Meier R., and F. Kradolfer. 1956 Schutzwirkung von Polysaccharidartigen Fraktionen bakterieller Herkunft bei einer experimentellen Virusinfektion. Experientia 12 13—214
- 86 Merckenschlager M. K. Schlossmann and W. Kurz. 1957 Ein mechanischer Zellhomogenisator und seine Anwendbarkeit auf biologische Probleme. Biochem. Z. 329 332—340
- 87 Miller C. P. C. W. Hammond, M. Tompkins, and G. Shorter. 1952. The treatment of postirradiation infection with antibiotics. An experimental study on mice. J. Lab. Clin. Med. 39 46—479
- 88 Mollaret P. 1950 Ueber Nutzen und Gefahren des Chloramphenicols (Chloromycetin) bei der Behandlung des Typhus und Paratyphus. Wien klin. Wochschr. 62 381—384
- 89 Mourad, S. and L. Friedman. 1961 Active immunization of mice against *Candida albicans*. Proc. Soc. Exptl. Biol. Med. 106 570—572
- 90 Mundkur B. 1960 Electron microscopic studies of frozen-dried yeast. I. Localization of polysaccharides. Exptl. Cell Research 20 28—42
- 91 Munoz, J. E. Ribó, and C. L. Larson. 1959 Antigens of *Beauveria perniciosa*. II Activities of cell walls and protoplasm. J. Immunol. 133 496—501
- 92 Nagata A. 1962. Studies on the fine structure of *Candida albicans* (In Japanese) Yonago Igaku Zasshi (J. Yonago Med. Assoc.) 13 4—24
- 93 Negroni, P. 1936 Étude de la capsule de *Mycotorula albicans* (Ch. Robin, 1853). Ann. parasit. l. humaine et comparée 14 511—516
- 94 Nemes, M. M. and M. R. Hilleman. 1962 Effect of Westphal lipid A on viral activities in mice and hamsters. Proc. Soc. Exptl. Biol. Med. 110 500—504
- 95 Nickerson W. J. and G. Falcone. 1959 Function of protein disulfide reductase in cellular division of yeasts, p. 409—424. I. R. Benesch, (ed.) Sulfur in proteins. Proceedings of a symposium held at Falmouth, Mass. May 1958. Academic Press, New York.
- 96 Nisani, C., and C. Frisipaldi. 1954 Allergie et réactions d'hypersensibilité en immunité au cours des infections expérimentales par les mycètes blastospores (Not IV). Boll. vet. ital. soc. intern. microbiol. 6 474—479
- 97 Northcote D. H. and R. W. Horne. 1952 The chemical composition and structure of the yeast cell wall. Biochem. J. 51 232—236
- 98 Orth, E. and O. Runzicka. 1950 Untersuchungen am Elektronenmikroskop über die Chloromycetin-Wirkung. Wien. med. Wochschr. 100 570—571
- 99 Ostrovsky F. 1896 Recherches expérimentales sur l'infection générale produite par le champignon d'ergot. Parallèle pathogénique entre la maladie mycotique et l'infection bacté-

- rence. Medical thesis, Paris G Steinhell. Paris 70 p.
- 100 Owen, C. R., M. B. Anderson, and A. T. Henrici. 1937 Allergy in Monilia and yeast infections. *Mycopathologia (The Hague)* 1: 10-25.
  - 101 Price, T. F. 1952. *In vivo* experiments with *Monilia* and *Escherichia coli* to explain moniliasis in patients receiving antibiotics. *Antibiotics & Chemotherapy* 2: 653-658.
  - 102 Philipson, J. 1957 Experimental studies on enhanced resistance to infection following some non-specific measures. *Acta Pathol. Microbiol. Scand. Suppl. N* 32: 1-148.
  - 103 Pienert, W. 1955 Studie zum Verhalten von *Candida* in der Kinderklinik. *Deut. Gesundheitsw.* 10: 1374-1380.
  - 104 Popfki, L., and Z. Vlasin. 1960. Zur Frage der serologischen Untersuchung bei oberflächlichen *Candida*-Infektionen. *Dermatologica* 120: 223-230.
  - 105 Prochazow, J. J. 1962. Treatment of opportunistic fungus infections. *Lab. Invest.* 11: 1217-1230.
  - 106 Ramado Guerra, A. U. J. L. Agostto C. Fossati, A. Ferreira Apolo, F. Paeyre, J. E. Mackinnon, and R. C. Ariagaveytas-Alende. 1937 Hipogamaglobulinemia congenita familiar con moniliasis disseminada grave familiar y eubacterias atipicas. *Rev. colombiana pediatr. puercult.* 16 (special number): 102-115.
  - 107 Rabin, H. A. D. Rowley C. Jenkins, and J. Fine. 1960. On absorption of bacterial endotoxin from the gastrointestinal tract of the normal and shocked animal. *J. Exptl. Med.* 112: 783-792.
  - 108 Reinman, H. A. and T. J. Kurotchkin. 1931 Attempts to produce bronchomycosis in monkeys. *Am. J. Trop. Med.* 11: 131-133.
  - 109 Ribi, E. K. C. Milner and T. D. Furrie. 1959 Endotoxin and antigenic fractions from the cell wall of *Salmonella enteritidis*. Methods for separation and some biologic activities. *J. Immunol.* 82: 75-84.
  - 110 Rimbaud P. J.-A. Rioux, and J. M. Bastide. 1960. Les phénomènes immunologiques dans les candidoses les réactions sérologiques. *Bull. soc. franç. dermatol. syphilig.* 67: 673-684.
  - 111 Roger M. 1896 Modification du sérum chez les animaux vaccinés contre l'aldidrie albicans. *Compt. rend. soc. biol.* 48: 720-730.
  - 112 Roger M. 1896. L'infection aldisienne. *Presse méd.* 6: 105-109.
  - 113 Rosebury T. D. Ogle and D. F. Taylor. 1954 An approach to the study of interactive phenomena among microorganisms indigenous to man. *J. Bacteriol.* 67: 135-152.
  - 114 Rosen, F. S. 1961 The endotoxins of gram-negative bacteria and host resistance. *New Engl. J. Med.* 264: 919-923, 980-985.
  - 115 Roth, F. J. J. and W. H. Murphy J. 1957 Lethality of cell-free extract of *Candida albicans* for chlortetracycline-treated mice. *Proc. Soc. Exptl. Biol. Med.* 94: 530-532.
  - 116 Rowley D. (1955. Rapidly induced changes in the level of non-specific immunity in laboratory animals. *Brit. J. Exptl. Pathol.* 37: 223-234.
  - 117 Rubenberg, S. H. F. H. Schweinsburg, and J. Fine. 1960. *In vitro* deactivation of bacterial endotoxin by macrophages. *J. Exptl. Med.* 112: 801-807.
  - 118 Rubenberg, S. H., H. E. Smith, A. M. Rubenberg, and J. Fine. 1962. Degradation of endotoxin by splenic extracts. *Antimicrob. Agents & Chemotherapy (Detroit)* 1961: 142-147.
  - 119 Ruzicka, O. 1951 Elektromagnetisch fassbare Wirkung der Antibiotika. *Monatsschr. Kinderheilk.* 99: 22-24.
  - 120 Ruzicko, L. A. Stachow A. Nowakowska, and J. Kubica. 1958. Chemical and biological properties of cell walls of *Candida krusei*, *Trichophyton sppsuum* and *Penicillium notatum*. *Bull. acad. polon. sci. Classe II.* 6: 15-20.









# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 420

## EFFECT OF DIGITALIS GLUCOSIDES ON ELECTROCARDIOGRAM AND EXERCISE TEST IN HEALTHY SUBJECTS

By

GUNVOR NORDSTRÖM-ÖHRBERG

ACCOMPAN

---

STOCKHOLM 1964

# ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of *Nordiskt Medicinskt Arkiv* founded in 1869 by Axel Key. The first volume of *Acta Medica Scandinavica* is therefore numbered LII (52).

The chief editors have been Axel Key 1869—1900, C. G. Santesson 1901—1915, I. Holmgren 1916—1937 and Birger Strandell 1938 to date.

*Acta Medica Scandinavica* publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board. Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the Editor at the address below.

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that, if accepted, it will not be republished in any other journal in the same or similar form, without the Editor's written consent.

Papers will be published in English, French or German at the authors' choice, followed by a summary in English.

The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left hand margin.

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author. No paper shall exceed 24 printed pages.

## Subscription

The annual subscription to the journal, covering two volumes, each of 6 numbers is 140 Sw. crowns or U.S. \$ 27.25 including postage in the Scandinavian countries and in Holland 120 Sw. crowns.

*Address for subscriptions and all communications*

ACTA MEDICA SCANDINAVICA

P. O. Box 2052 Stockholm 2

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

From the Department of Clinical Physiology  
and  
the Department of Medicine  
of Karolinska Institutet at Serafimerlasarettet, Stockholm, Sweden

EFFECT OF DIGITALIS GLUCOSIDES ON  
ELECTROCARDIOGRAM AND EXERCISE TEST IN  
HEALTHY SUBJECTS

BY  
GUNVOR NORDSTRÖM-ÖHRBERG

STOCKHOLM 1964

Boktryckeriaktiebolaget Thule  
Stockholm 1964

# CONTENTS

INTRODUCTION	5
MATERIALS AND METHODS	8
RESULTS I	13
Subjective symptoms	13
Effect on heart rate	15
Effect on certain aspects of circulatory functions and dimensions	20
Discussion	30
RESULTS II	35
Electrocardiographic changes	35
Method of measurement	35
Effect on rhythm and conduction	38
Effect on the ST and T region	45
Discussion	60
Duration of ECG changes	66
REFERENCES	69
SUMMARY AND CONCLUSIONS	73
ACKNOWLEDGEMENTS	75



Boktryckeraktiebolaget Thulc  
Stockholm 1964

## INTRODUCTION

Ever since Withering published *An Account of the Foxglove and some of its Medical Uses* in 1785 the haemodynamic effect of the digitalis glucosides both on healthy persons and on patients with cardiac insufficiency has been the subject of extensive study. Withering's lucid thought, his brilliant style and the rich variety of his observations have however hardly been surpassed in any clinical account during the subsequent centuries.

The conception of the mode of action of the digitalis glucosides has changed considerably in the course of the years, as also whether their primary point of attack is the myocardium, the peripheral blood vessels or the kidney. Whereas there always seems to have been a consensus of opinion concerning the favourable effects of digitalis on the failing heart, the opinions on its effect on the healthy heart have greatly varied. Early reference was made to the paradoxical effect of the cardio-active glucosides on the normal circulation.

The measure of this harmful effect seems to be a lowering of the cardiac output (Stewart & Cohn 1932, MacMichael & Sharpey-Schafer 1944, Bing et al. 1950, Harvey et al. 1951, Williams et al. 1958). By increasing the diastolic tone of the heart, digitalis would appear to prevent its normal filling during diastole so resulting in smaller cardiac output (Stewart & Cohn 1932). Another view which was first advanced in conjunction with animal experiments by Dock & Talbot in 1930 and in 1944 after experiments on man by MacMichael & Sharpey

Schafer was that the primary cause of the reduced cardiac output was a lowering of the central venous pressure, that in fact the primary effect of the cardioactive glucosides was to be sought on the venous side, an opinion which Lagerlöf & Werkö (1949) proved to be untenable.

The mechanical efficiency of the heart was also thought to decrease in healthy subjects (Erickson & Fahr 1945, Bing et al. 1950). "Digitalis acted as a cardiac poison" (Erickson & Fahr).

This negative attitude to the effect of the digitalis glucosides on the healthy or non-insufficient heart soon changed. As early as 1924 Luten had presumed that digitalis increased the muscular efficiency of the heart muscle. Later in experiments on the isolated mammalian heart, it was shown that digitalis increases the force of contraction of the heart (Wiggers & Stimson 1927, Cautell & Gold 1938). That this applies also to the intact heart has since been proved by several authors (Walton et al. 1950, Cotten & Stopp 1958, Braunwald et al. 1961). According to this view digitalis stimulates the force of systolic contraction of the healthy heart, with consequent increase of blood pressure as an unchanged peripheral resistance. The increase in force of contraction need in no way be reflected in changes of cardiac output. In actual fact several investigators have shown that digitalis exerts no haemodynamic action on the healthy or non-insufficient heart (Lijstrand & Nylin 1941, Lagerlöf & Werkö 1949, Werkö et al. 1958, Dresdale et al. 1959, Seher et al. 1959). In acute-phase experiments with lanatoside C, Rodman et al. (1961) showed a transient reduction of the resting

Their paper contains an excellent bibliography of the older literature on the subject.



intravenously into 20 young healthy persons. The exercise was light judging from the heart rate. It was done on a treadmill and one chest lead was recorded. Yu et al., too, found ST-T changes which at times were extremely pronounced.

These three studies, which appear to be the only ones relating to the effect of digitalis on the exercise ECG have comprised few observations and a relatively unstandardized test in which the exercise was quite light. In the only study dealing with the ECG reaction also during exercise (Yu et al.) the system of ECG recording was unsatisfactory.

In view of the very incomplete study of the effect of the digitalis glycosides on the circulatory response and on the ECG reaction during muscular exercise, these problems have been taken as the subject of the present study. For this purpose the form of exercise has been used which was introduced by Sjöstrand in 1947 and has since been employed in several other Swedish investigations (Wahlund 1948, Holmgren 1956, Bengtsson 1956 b, Ljunroth 1957, Sandberg 1957 and 1961 and others).

cardiac output in young healthy subjects this effect lasted about one hour. The same transient effect was evident from the study made by Harvey et al. (1951) who found the cardiac output to be reduced within one hour but thereafter restored.

The effect of digitalis during exercise has been studied to a comparatively little extent. In 1952 Yu et al. showed that light exercise on a treadmill could be performed at a lower heart rate after digitalization, while in 1958 Nordstrom-Öhrberg reported that acute-phase administration of cardioactive glucosides did not affect the working capacity or certain circulatory parameters in healthy subjects. Determination of cardiac output during exercise was done by Williams et al. (1958) in acute phase experiments. They found a lowered cardiac output. The experiments were made within one hour of digitalization and accorded in time with the transient effect on the cardiac output observed by Rodman et al. (1961) who however in experiments lasting two days, found no influence on the cardiac output either at rest or during exercise. Schröder et al. (1962) arrived at the same results in experiments with prolonged digitalization of healthy persons.

Digitalis glucosides have an effect also on the peripheral vascular bed. It was found at an early stage that digitalis provoked a rise of pressure in the systemic circulation (Dock 1930 Katz et al. 1938). Later Ross et al. (1960) made an extensive study of the effects of digitalis on the peripheral circulation, which revealed that the earlier observed rise of blood pressure was caused partly by a raised peripheral resistance owing to a direct effect on the smooth musculature in arterioles. This increase of the peripheral resistance sets in before the positive inotropic effect.

The effect of digitalis on the resting electrocardiogram (ECG) of healthy per-

sons had been observed early on (Cohn & Fraser 1913 Pardee 1923 Routier & Puddu 1935 Larsen & al 1937 Geiger et al 1941 Dönhardt 1951 Koefling 1950 Tilakos 1953 and others). It has been constantly suggested that the ECG changes might be an indicator of the degree of digitalization.

The value of electrocardiography in conjunction with muscular work for the diagnosis of ischaemic heart disease is well documented. In Sweden stress has been placed on the importance of using a form of exercise and location of the electrodes which allow electrocardiography also during exercise (Bengtsson 1936 a, Sandberg 1957 and 1961 I Åstrand 1960). This increases the safety of the test for the patient and the accuracy of the electrocardiographic diagnosis.

It is imperative that different sources of error in the exercise test should be analysed in order that the diagnosis may be as certain as possible. Pharmacological preparations, especially the digitalis glucosides constitute an important source of error.

Very little information has been published concerning the electrocardiographic reaction to digitalis during exercise.

Zwillingner (1935) found changes in the ST and T segments after exercise also in healthy persons who had received digitalis. Liebow & Feil (1941) studied the electrocardiographic reaction after exercise on 14 young healthy subjects. Digitalis was administered per os until signs of intoxication appeared. Apart from lower heart rate and isolated cases of arrhythmia, a general finding was a depression of the ST segment and the authors maintain that it is impossible to distinguish the digitalis effect from the effect seen in coronary disease.

The effect of digitalis on the ECG during exercise has earlier been studied only by Yu et al. (1952) who injected digoxin

### *O<sub>2</sub> consumption*

The O<sub>2</sub> consumption was calculated after analysis of the content of O<sub>2</sub> and CO<sub>2</sub> in the expired air ad modum Krogh-Haldane. The standard error of a single determination calculated from duplicate determinations and expressed as coefficient of variation was 0.6 % of the mean for CO<sub>2</sub> and 0.13 % for O<sub>2</sub> (n = 200).

The error of the method for the ventilation determined on separate days was at rest about 15 % and at the highest loads about 7 %. For duplicate determinations at the same examination the error of the method was at rest 5 % and during exercise 10 %. These values are of the same order of magnitude as reported earlier (cf I Åstrand, 1960 and Holmgren and Pernow 1958).

### *Mechanical efficiency*

The O<sub>2</sub> uptake was measured at rest and was used for calculation of the mechanical efficiency. The mechanical efficiency was also calculated from predicted value (cf I Åstrand 1960). The two values of mechanical efficiency are compared in the account of the results. A caloric coefficient for oxygen of 4.825 cal/lit was used at rest and of 4.900 during exercise.

### *Lactate*

Samples for lactate determination were collected from the fingertip at rest, at the 6th minute at each load, and 2 minutes after the end of exercise. The blood was precipitated with trichloroacetic acid within 30 seconds. The analyses were made by Ström's modification (1949) of the method of Barker and Summerson (1941). The error of the method, calculated from duplicate determinations on the same blood sample was 4.4 % at a mean concentration of 1.42 mM/l, 2.1 % at a mean concentration of 6.5 mM/l and

2.2 % at a mean concentration of 9.5 mM/l. The error of the analysis was of the same order of magnitude as reported by Holmgren, (1956) Carlson and Pernow (1959) and I. Åstrand (1960). This method of accounting for the error of the method, accordingly does not reveal the sampling error. The error of the method is considerably larger if duplicate determinations are made on the same individual on different days and at separate examinations.

### *Total amount of haemoglobin*

The total haemoglobin was determined by the liveolar carbon dioxide method originally devised by Sjöstrand (1948) and modified in certain respects by Wiklander (1956). The standard error of the single determination calculated from duplicate determinations was 4 % at this laboratory. The haemoglobin concentration, Hb (g/100 ml) was determined by haemolysing blood in 0.04 % ammonia. The density of the solution was measured in Beckman B spectrophotometer. Blood samples were collected by pricking of the fingertip. The error of the method was about 4 % including the sampling error.

The blood volume was calculated from total haemoglobin and haemoglobin concentration.

### *Heart volume*

The heart volume was determined in prone position by the method of Larsson and Kjellberg (1948) and Kjellberg et al. (1949) with exposure in two planes without special regard for the contraction phases of the heart. Kjellberg et al. (1951) showed that the effect of the heart cycle on heart size in the recumbent body position is negligible except possibly when the pulse rate is very low. By this method the posteroanterior projection is taken with

## MATERIAL and METHODS

### Material

A total of 92 tests were made on 64 healthy volunteers: 32 men aged 17–55 years, average 28 years, and 32 women aged 20–55 years, average 35 years. They were mostly physicians, medical students and nurses. None had a history of cardiac or vascular disease and none exhibited any sign of illness at the clinical examination. All were in full time employment.

### Methods

#### *Electrocardiography*

Electrocardiograms (ECG) were recorded with a direct writing four-channel apparatus (Mingograph 42 Elema, Stockholm). The paper speed was 50 mm per second. The electrodes were plates of 30 mm diameter. Eight leads were used for recordings at rest, in standing and after exercise: viz. standard leads I, II, III and CR leads 1, 2, 4, 5 and 7. During exercise the indifferent electrode was moved to the forehead the CH lead. In normal cases CH and CR leads may be considered practically identical (Bengtsson 1956 c, Holmgren and Strandell 1961). The sensitivity was 10 mm for 1 mV.

#### *Orthostatic test*

The exercise test was routinely preceded by an orthostatic test (Sandberg 1957, 1961, Holmgren et al. 1957 and 1959). The subjects stood for 8 minutes as relaxed as possible with the head leaning against a wall, after which the ECG was recorded.

#### *Exercise test*

The exercise test was performed according to the method elaborated by Sjöstrand (1947), Wahlund (1948), Holmgren (1956) and others. The work was performed at successively rising loads on an electrically braked bicycle ergometer designed by Holmgren and Mattsson (1954). The subjects cycled for 6 minutes at each load starting at 300 kpm/min, then 600, 900 and possibly 1200 for men, and 200, 400, 600, 800 kpm/min for women. ECG records were taken during the test at the 2nd, 4th and 6th minute at each load, and immediately 3 minutes and 10 minutes after the end of the exercise. The same bicycle ergometer was used throughout the experimental series. It was calibrated annually but no major deviations were noted. The test was as a rule continued up to a heart rate of about 170 beats a minute (Robbe and Ström 1958, Hellström 1961 and others).

All heart rates were counted from the ECG tracings. The respiratory rate was counted by auscultation at rest, during the 5th minute at each load and after the completion of exercise. The working capacity ( $W_{170}$ ) has been defined as the work performed at heart rate 170 beats/min.

#### *Ventilation*

The total ventilation was determined by collection of the expired air in a Douglas bag for 5 minutes at rest and during the 3rd–5th minutes at each load. The gas volumes were measured in a gasometer. The breathing rate was counted for 1 minute in the middle of each collecting period.

Through the choice of lanatoside C, digitoxin and digoxin the study has comprised one compound with rapid effect and good solubility (*lanatoside C*) one with prolonged cumulative effect (*digitoxin*) and one peroral compound with rapid resorption capacity and brief effect (*digoxin*).

The drugs were given both in therapeutic and in toxic doses. Thus *lanatoside C* was given in doses of 0.4 and 0.8 mg intravenously and of 1.6 mg intravenously the latter may be regarded as an average digitalization dose. This dosage implies that some subjects become fully digitalized, others not. In the case of *digoxin* a single dose of 1.25 mg per os was used, which is the average digitalization dose for this glycoside. In the case of *digitoxin* doses which produced signs of intoxication in more than half of the subjects (1.2—3.6 mg) were used. These doses of digitalis are largely in accordance with those proposed by previous authors.

## Procedure

A clinical evaluation of the subjects was made before the digitalis tests. This evaluation included in all cases medical history, clinical examination, determination of haemoglobin concentration, sedimentation rate, and urinary examination for protein, sugar and casts. X-rays were taken of heart and lungs, and total haemoglobin was determined. ECG records were taken resting, standing, and during and after exercise. In the older age group determinations were made also of serum cholesterol, in some cases, too, of serum iron.

The subjects were not in basal state and took a light morning meal on the days when the tests were made. All exercising electrocardiograms were made at the

same time of the day about 2 hours after a light meal.

### 1 Tests with *lanatoside C*

#### a) 1.6 mg intravenously

All digitalization was done in the morning. A resting ECG was recorded after about 15 minutes of rest and *lanatoside C* was then slowly injected intravenously. The injection generally took 5 minutes. The total dose was 1.6 mg, which was generally divided into two doses of 0.8 mg injected with half-an-hour's interval. This was done because some subjects who were given a single dose of 1.6 mg had pain in the arm even if the injection was given slowly. No inconvenience was suffered when the dose was divided. After the injection, electrocardiograms were recorded at rest at 15-minute intervals for about 1 hour. About 3 hours after the last injection the exercise test was started. Thereafter a heart X-ray was taken, followed by determination of total haemoglobin. The subject returned daily to the laboratory always at the same time in the afternoon, and daily ECG records were taken resting, standing, during and after exercise as long as ECG changes persisted. This group comprised 21 men aged 22—55 years, average 29 years, and 21 women aged 21—54 years, average 32 years.

#### b) 0.4 and 0.8 mg *lanatoside C* intravenously

The procedure was largely the same as for the 1.6 mg test. The subjects were injected with 0.4 mg *lanatoside C* intravenously in the morning. Three hours later ECG was recorded resting, standing, and during and after exercise. The same procedure was repeated a week later this time after injection of 0.8 mg *lanatoside C* intravenously. This group comprised 11 subjects, 6 men aged 17—33 years, aver



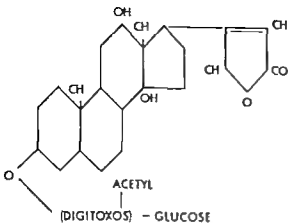


Fig. 1

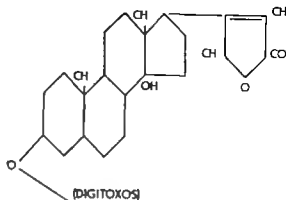


Fig. 2

the tube angled  $30^\circ$ . The error of the method according to Kjellberg *et al.* (1951) is about 4%. The method used in the present study differs from the above-mentioned in the respect that we did not use an angled tube and the heart volume was calculated according to Jonsson (1939). By the method of heart size determination in recumbent position employed at the Serafiner Hospital the error is about 4%. The heart volume was also determined in standing position.

### Blood pressure

The blood pressure was measured in the right arm recumbent at heart level and after 8 minutes standing by the auscultatory cuff method. The diastolic pressure was taken to be the pressure when the Korotkoff sounds disappeared.

### Choice of digitalis drugs

Cardioactive glucosides is the collective name for a group of substances in nature with the same basic properties in respect of effect and chemical structure. At present there is no synthetic digitalis compound. The pharmacological effect of a cardiac glucoside lies in the aglucone, while properties such as solubility, dura-

tion of effect, etc. lie in a sugar molecule, usually a monosaccharide.

Digitalis drugs with varying time of onset and duration of effect were chosen for this study. They have also been thoroughly investigated in respect of their pharmacological and clinical effects and of their resorption and excretion conditions. They have all been put to wide clinical use.

*Lanatoside C* (Cedilanid(R) Sandoz) is one of the three glucosides that has been isolated in *digitalis lanata*. The aglucone in lanatoside C consists of digoxigenin; the molecule also contains three molecules of digitoxose, one molecule of glucose and one molecule of acetic acid (Fig. 1). Cedilanid has been injected intravenously.

Digoxin (ACO) has been isolated both from *digitalis lanata* and *digitalis purpurea*. The cardioactive aglucone consists of digoxigenin with three molecules of digitoxose, glucose and acetyl as radicals (Fig. 2). Digoxin has been given perorally.

Digoxin (Lanacrist Draco) is in principle the same cardioactive glucoside as lanatoside C, but with one glucose molecule and one acetyl group less. Digoxin may thus be regarded as the pure gluconide from lanatoside C. Digoxin has been given perorally.

## RESULTS I

### Subjective symptoms

#### *General considerations*

A large variety of subjective symptoms in conjunction with digitalis medication have been described in the literature. The symptoms noted have been gastrointestinal such as a feeling of discomfort in the epigastrium, increased salivation, anorexia, nausea, vomiting and diarrhoea; neurological such as headache, visual disorders (scotoma, yellow-green vision, double vision); general symptoms such as fatigue, perspiration; cardiac symptoms such as palpitation (actually a feeling that the heart is beating hard) and symptoms due to disorders of impulse formation and conduction disorders (Winthering 1783, Herrmann et al., 1944, Burwell et al., 1950, Cohen, 1952 a, b, and others).

Rarer symptoms are objectively observable endocrinological disorders such as gynecomastia (Le Winn 1953). The gastrointestinal symptoms are by far the most common and occur not only in response to galenicals but also to purified drugs.

The best early description of the appearance and course of these symptoms is that by Winthering (1783) who wrote

The Fougloze when given in very large and quickly-repeated doses, occasions sickness, vomiting, purging, giddiness, confused vision, objects appearing green or yellow, increased secretion of urine, the frequent motions to part with it, and sometimes inability to retain slow pulse, even as slow as 35 in violent cold sweats, convulsions, syncope, death.

When given in less violent manner it produces most of these effects in lower degree.

Subjective symptoms are considered to

constitute a certain measure of digitalization, from "full digitalization" to intoxication. Toxic signs are therefore regarded as extremes of the therapeutic effect (Wright 1960).

#### *A. Symptoms following intravenous injection of lanatoside C*

Nine subjects were given 0.4 mg of lanatoside C intravenously. None had subjective symptoms.

Eight subjects were given a dose of 0.8 mg intravenously. One subject had nausea and became tired about two hours after the injection. The symptoms lasted about half-an-hour. Otherwise no subjective symptoms appeared.

Forty-two subjects were injected with 1.6 mg of lanatoside C, 12 of whom developed subjective symptoms (table 1A). The symptoms generally appeared 1½–2 hours after the injection. All were able to perform an exercise test although one (no. 27) broke off the test at a lower load than before the medication. The symptoms were comparatively mild and of brief duration. The nature of the symptoms is shown in table 1. One subject (no. 27) had more pronounced symptoms lasting several hours. It will be seen from the table that the dose per kg body weight varied from 0.018 to 0.031 mg. The mean dose 0.025 mg/kg bodyweight was the same as for those who had no symptoms. The dose per kg body weight was therefore of no significance. Almost all subjects who were given 1.6 mg lanatoside C thought that they perspired more during the exercise test, but only the definite cases are included in the table.

age 22 years, and 3 women aged 20—51 years, average 33 years.

## 2 *Digitoxin per os*

Digitoxin was given per os for two to ten days in a total dose of 1.2—3.6 mg. On the appearance of nausea or serious ECG changes, the medication was stopped. With a few exceptions the digitoxin tablets were administered daily in the laboratory in order to ensure that the subjects actually took them. During the digitalization resting electrocardiograms were recorded daily. On the last day or one day after the digitalization the procedure was repeated with exercise test and determination of heart volume and total haemoglobin. In ten cases determinations were made also of the lung ventilation, oxygen consumption and of the lactate concentration during and after exercise, all in the same order as in the tests with 1.6 mg of lanatoside C intravenously.

After the course of digitalization, ECG at rest, standing and in conjunction with exercise were recorded once a week as long as ECG changes persisted. This group comprised 23 subjects: 11 men aged 22—

55 years, average 30 years, and 12 women aged 21—55 years, average 36 years.

## 3 *Digoxin per os*

A single dose of 1.25 mg digoxin (Lanacrist(R)) was given per os in the laboratory in the morning after a light meal. The subjects kept in contact with the laboratory during the day for check of subjective symptoms etc. Five hours later orthostatic and exercise ECG were recorded. This procedure was repeated daily until ECG changes had disappeared. This group comprised ten subjects, 8 men aged 23—37 years, average 30 years, and 2 women aged 21—32 years, average 37 years.

## Statistical methods

Conventional statistical methods have been used (Fisher 1950; Kemp & Nielsen 1959).

Significance was tested by the *t*—test.

Levels of significance

$p = 0.05$ — $0.01$  probably significant

$p = 0.01$ — $0.001$  significant

$p < 0.001$  highly significant

### B. Symptoms following administration of digitoxin

This series comprised 25 subjects, 13 of whom developed subjective symptoms (table 1 B). The subjective symptoms after digitoxin were more pronounced than after lanatoside C intravenously. The total dose varied from 1.2 mg to 3.6 mg per os spread over 2–14 days. Here again, accordingly there was no correlation between dose and symptoms. The symptoms generally appeared 8–12 hours, on one occasion 3 hours, after the last dose. Their duration was 2–3 hours.

### C. Symptoms following administration of digoxin

Ten subjects were given single dose of 1.25 mg digoxin. Eight of them had subjective symptoms, as shown in table 1 C, in all cases consisting of nausea, in two combined with vomiting. The symptoms appeared 40 minutes to 2 hours after medication and were of comparatively brief duration. All subjects were able to carry out the exercise test.

### Summary

The incidence and nature of the subjective symptoms in conjunction with digitalis medication in healthy volunteers is reported. The cardioactive glucosides were administered partly intravenously partly per os.

Lanatoside C in doses of 0.4–0.8 and 1.6 mg was used as parenteral glucoside. No subject developed symptoms after 0.4 mg, only one after 0.8 mg, and even after 1.6 mg the subjective symptoms were comparatively rare, of a mild nature and rapidly disappeared. In those who developed symptoms the threshold dose per kg body weight varied greatly and did not differ from that administered to subjects who had no symptoms.

The subjective symptoms were more common, more pronounced and of longer duration after oral administration of digitoxin. The dose after which symptoms appeared again varied, from a total of 1.2 to 3.6 mg spread over 2–14 days. No subjective symptoms were reported after smaller doses than 1.2 mg.

After a single dose of 1.25 mg digoxin, symptoms were common and fairly pronounced, and appeared soon after the medication, as opposed to the other oral preparation, digitoxin. The symptoms rapidly disappeared.

### Effect on heart rate

#### 1. Heart rate at rest

##### A. Lanatoside C

The resting heart rate for men was  $66 \pm 2.3$  S.D. 11 ( $n = 21$ ) after injection of 1.6 mg of lanatoside C intravenously. The difference in rate before and after digitalis is not significant ( $p = 0.5-0.4$ ) (table 5).

For women the corresponding values were  $72 \pm 2.5$  S.D. 12 ( $n = 21$ ) before and  $66 \pm 3.2$ , S.D. 15 ( $n = 21$ ) after lanatoside C. Here the rate is rather lower and the difference probably significant ( $p = 0.05-0.01$ ). If men and women are taken together there is no significant difference ( $p = 0.2-0.1$ ).

Lanatoside C was given also in smaller doses, 0.4–0.8 mg. The heart rate before administration was  $68 \pm 1$ , S.D.  $\pm 11$  and after  $65 \pm 2.0$  S.D.  $\pm 8$  ( $n = 17$ ). The difference before and after these small doses of lanatoside C is not significant ( $p = 0.3-0.2$ ).

##### B. Digitoxin

This series (table 6) comprised 25 subjects, of whom 11 men and 12 women, who received digitoxin tablets per os for

Table 1

## A. Subjective symptoms after single dose of 1.6 mg lanatoside C intravenously

Subject number	Symptoms	Dose mg/kg body weight	Time of onset of symptoms
2	Nausea and fatigue	0.023	1 h
3	Feeling of discomfort and fatigue	0.025	1½ h
5	Fatigue	0.019	2 h
8	Nausea, fatigue, perspiration	0.026	1 h
11	Feeling of discomfort in epigastrium	0.025	1½ h
15	Feeling of discomfort in epigastrium, fatigue, perspiration	0.018	2 h
19	Fatigue	0.029	1 h
20	Feeling of discomfort in epigastrium	0.031	1 h
25	Fatigue	0.020	2 h
27	Nausea, vomiting, fatigue, perspiration, dizziness	0.029	1½ h
31	Nausea, fatigue	0.031	2 h
40	Fatigue	0.028	1 h

## B. Subjective symptoms after digitoxin per os

Subject number	Symptoms	Dose mg	Approx. time in hours after last dose
11	Increased salivation	1.2	8 h
31	Nausea, vomiting, palpitation, fatigue	1.8	12 h
32	Nausea	1.6	6 h
34	Nausea, fatigue	1.2	8 h
37	Nausea, increased salivation, palpitation	1.8	ca 12 h
38	Fatigue	2.6	5-6 h
41	Palpitation, nausea	1.6	12 h
42	Anorexia, nausea	1.2	3 h
43	Palpitation, perspiration, epigastric discomfort, fatigue	1.6	12 h
45	Epigastric discomfort, fatigue	2.0	10-12 h
46	Nausea, perspiration, palpitation	3.6	10 h
47	Nausea, epigastric discomfort, fatigue, palpitation	1.4	8-10 h
55	Fatigue	2.8	

## C. Subjective symptoms after single dose of 1.25 mg digitoxin per os

Subject number	Symptoms	Dose mg/kg	Time of onset of symptoms
15	Nausea, vomiting	0.015	1 h
24		0.022	40 min.
35		0.016	1 h
39		0.022	1 h
54		0.017	1 h
56		0.020	1½ h
57	vomiting	0.017	2 h
51		0.019	1 h

To throw further light on this situation, further experiments were made in which 1.6 mg of lanatoside C intravenously and digitoxin orally were given to 14 subjects on different occasions.

These show the same pattern, a resting heart rate of 63 before digitalization, of 63 beats/min after lanatoside C and 63 beats/min likewise after digitoxin (table 3). The heart rate in standing was also higher after lanatoside C. The difference is probably significant ( $p = 0.02-0.01$ ). The orthostatic pulse reaction, i.e. the difference between recumbent and standing heart rate, increased after lanatoside C, the difference being significant ( $p = 0.01-0.001$ ).

After digitoxin, on the other hand, there was no significant difference either at rest ( $p = 0.4-0.3$ ) standing ( $p = 0.9-0.8$ ) or in respect of orthostatic pulse reaction ( $p = 0.4-0.3$ ).

In the lanatoside C and digitoxin groups there were subjects who had subjective symptoms of varying degree, and it is not unreasonable to imagine that they represent roughly the same degree of digitalization.

It is apparent from table 4 that the tendency is the same as before. The resting heart rates are lower for those who had subjective symptoms after digitalization, whether with lanatoside C or digitoxin. In both groups the difference was probably significant ( $p = 0.03-0.01$ ).

In the lanatoside C group without symptoms the orthostatic pulse reaction was 23 beats/min after digitalization against 13 before the difference in reaction is significant ( $p = 0.01-0.001$ ) and in the group with symptoms the reaction was still stronger.

Before lanatoside C the orthostatic pulse reaction was 17 and after 33 beats/min ( $p < 0.001$ ) in this latter group.

In the symptomatic group the standing

heart rate before lanatoside C was 88 beats/min, after digitalization 99 beats/min. The difference is significant ( $p = 0.01-0.001$ ).

The subjects who had signs of intoxication after digitoxin had a rather lower resting rate (table 4) the difference being probably significant ( $p = 0.02-0.01$ ). But there were no significant differences in respect of standing heart rate and orthostatic pulse reaction.

### 3 Heart rate during exercise

#### A. Lanatoside C

The results are shown in table 5. It will be seen that the heart rate reactions during exercise before and after intravenous injection of lanatoside C were practically identical. At a load of 300 kpm/min the men had an average heart rate of 98 beats/min before and 99 after digitalization.

At the next load 600 kpm/min the corresponding heart rates were 126 and 127 beats/min. At 900 kpm/min the average rate was identical before and after lanatoside C, 157 beats/min, and likewise at 1200 kpm/min, 168 beats/min.

The women, whose resting heart rate was lower after digitalization, displayed no appreciable changes during exercise. At a load of 200 kpm/min their average heart rate was 107 beats/min before and 108 after lanatoside C. At a load of 400 kpm/min the mean values were, respectively 134 and 133 beats/min, at 600 kpm/min they were identical before and after 160 beats/min, respectively. Intravenous lanatoside C caused no significant change in heart rate during exercise.

#### B. Digitoxin

The pulse reaction during exercise before and after digitoxin is shown in table 6.

doses varying between 1.2 mg spread over two days and 3.6 mg spread over 14 days. For men the heart rate before digitoxin was  $67 \pm 2.9$  S.D. 10 ( $n = 11$ ) and after digitoxin  $64 \pm 3.5$  S.D. 12 ( $n = 11$ ) i.e. very slightly lower. The difference was not significant ( $p = 0.3-0.2$ ). For women the corresponding values were  $66 \pm 3.2$ , S.D.  $\pm 11$  ( $n = 12$ ) before and  $62 \pm 3.0$  S.D. 10 ( $n = 12$ ) after. Here again the difference is not significant ( $p = 0.2-0.1$ ).

### C. Digoxin

After a single dose of 1.25 mg of digoxin per os to 10 subjects (6 men and 4 women) the heart rate before administration was  $68 \pm 3.6$  S.D.  $\pm 11$  ( $n = 10$ ) and after  $65 \pm 3.5$  S.D.  $\pm 11$  ( $n = 10$ ). The difference is not significant ( $p = 0.4-0.3$ ).

## 2 The orthostatic reaction

### A. Lanatoside C

The orthostatic pulse reaction (beats/min) i.e. the difference in heart rate between recumbent and standing position, was  $14.0 \pm 1.5$  S.D.  $\pm 7.4$  ( $n = 42$ ) before intravenous injection of 1.6 mg of lanatoside C and  $25.8 \pm 2.3$  S.D. 15 ( $n = 42$ ) after (table 2). Digitalization by lanatoside C thus brought a marked orthostatic reaction, the difference being highly significant ( $p < 0.001$ ).

A similar result was obtained with the lower doses of lanatoside C. Before digitalization the orthostatic reaction was  $16.3 \pm 3.2$  S.D. 10. The corresponding values after digitalization were  $25.3 \pm 2.8$  S.D. 11.4. Again the difference is highly significant ( $p < 0.001$ ) ( $n = 17$ ).

### B. Digitoxin

The orthostatic pulse reaction before digitoxin was  $16.3 \pm 2$  S.D.  $\pm 10$  ( $n = 23$ )

and after  $15.7 \pm 1.9$  beats/min S.D.  $\pm 9.0$ . There is thus no difference before and after digitoxin per os ( $p = 0.6-0.5$ ).

### C. Digoxin

In the digoxin group the orthostatic pulse reaction before medication was  $12.9 \pm 1.7$  S.D.  $\pm 5.4$  beats/min and after a single oral dose of 1.25 mg  $14.6 \pm 3.5$  S.D. 11. Here again there is no difference ( $p = 0.6-0.5$ ).

## Comparison between different groups

Table 2 contains data of the entire material before digitalization and after different doses of lanatoside C and digitoxin.

At rest the lowest values of heart rate were obtained after digitoxin. The difference is fairly small however about 5 beats/min, though probably significant ( $p = 0.05-0.02$ ). The other drugs showed no significant difference compared with the predigitalization figures ( $p = 0.6-0.5$ ). Considering the heart rates in standing the lanatoside C groups differ from the others, i.e. the standing rate is higher about 90 beats/min compared with 78 after digitoxin and 79 after digoxin. Before administration the heart rate was 83 beats/min.

The rise in heart rate after lanatoside C is probably significant ( $p = 0.05-0.01$ ) after the two other glucosides there was no significant difference in standing position. The orthostatic pulse reaction is most affected after lanatoside C as had appeared earlier: the difference is highly significant ( $p < 0.001$ ). There is undeniably significant difference in standing position. C in the standing position than to digitoxin and digoxin.

To throw further light on this situation, further experiments were made in which 1.6 mg of lanatoside C intravenously and digitoxin orally were given to 14 subjects on different occasions.

These show the same pattern, a resting heart rate of 63 before digitalization, of 63 beats/min after lanatoside C and 63 beats/min likewise after digitoxin (table 3). The heart rate in standing was also higher after lanatoside C. The difference is probably significant ( $p = 0.02-0.01$ ). The orthostatic pulse reaction, i.e. the difference between recumbent and standing heart rate, increased after lanatoside C, the difference being significant ( $p = 0.01-0.001$ ).

After digitoxin, on the other hand, there was no significant difference either at rest ( $p = 0.4-0.3$ ) standing ( $p = 0.9-0.8$ ) or in respect of orthostatic pulse reaction ( $p = 0.4-0.3$ ).

In the lanatoside C and digitoxin groups there were subjects who had subjective symptoms of varying degree, and it is not unreasonable to imagine that they represent roughly the same degree of digitalization.

It is apparent from table 4 that the tendency is the same as before. The resting heart rates are lower for those who had subjective symptoms after digitalization, whether with lanatoside C or digitoxin. In both groups the difference was probably significant ( $p = 0.05-0.01$ ).

In the lanatoside C group without symptoms the orthostatic pulse reaction was 23 beats/min after digitalization against 15 before; the difference in reaction is significant ( $p = 0.01-0.001$ ) and in the group with symptoms the reaction was still stronger.

Before lanatoside C the orthostatic pulse reaction was 17 and after 33 beats/min ( $p < 0.001$ ) in this latter group.

In the symptomatic group the standing

heart rate before lanatoside C was 88 beats/min, after digitalization 99 beats/min. The difference is significant ( $p = 0.01-0.001$ ).

The subjects who had signs of intoxication after digitoxin had a rather lower resting rate (table 4); the difference being probably significant ( $p = 0.02-0.01$ ). But there were no significant differences in respect of standing heart rate and orthostatic pulse reaction.

### 3 Heart rate during exercise

#### A. Lanatoside C

The results are shown in table 5. It will be seen that the heart rate reactions during exercise before and after intravenous injection of lanatoside C were practically identical. At a load of 300 kpm/min the men had an average heart rate of 98 beats/min before and 99 after digitalization.

At the next load 600 kpm/min the corresponding heart rates were 126 and 127 beats/min. At 900 kpm/min the average rate was identical before and after lanatoside C, 157 beats/min, and likewise at 1200 kpm/min, 168 beats/min.

The women, whose resting heart rate was lower after digitalization, displayed no appreciable changes during exercise. At a load of 200 kpm/min their average heart rate was 107 beats/min before and 108 after lanatoside C. At a load of 400 kpm/min the mean values were, respectively 134 and 133 beats/min, at 600 kpm/min they were identical before and after 160 beats/min, respectively. Intravenous lanatoside C caused no significant change in heart rate during exercise.

#### B. Digitoxin

The pulse reaction during exercise before and after digitoxin is shown in table 6.



doses varying between 1.2 mg spread over two days and 3.6 mg spread over 14 days. For men the heart rate before digitoxin was  $67 \pm 2.9$  S.D. 10 ( $n = 11$ ) and after digitoxin  $64 \pm 3.5$  S.D. 12 ( $n = 11$ ) i.e. very slightly lower. The difference was not significant ( $p = 0.3-0.2$ ). For women the corresponding values were  $66 \pm 3.2$  S.D.  $\pm 11$  ( $n = 12$ ) before and  $62 \pm 3.0$  S.D. 10 ( $n = 12$ ) after. Here again the difference is not significant ( $p = 0.2-0.1$ ).

### C. Digoxin

After a single dose of 1.25 mg of digoxin per os to 10 subjects (6 men and 4 women) the heart rate before administration was  $68 \pm 3.6$ , S.D.  $\pm 11$  ( $n = 10$ ) and after  $65 \pm 3.5$  S.D.  $\pm 11$  ( $n = 10$ ). The difference is not significant ( $p = 0.4-0.3$ ).

## 2. The orthostatic reaction

### A. Lanatoside C

The orthostatic pulse reaction (beats/min) i.e. the difference in heart rate between recumbent and standing position, was  $14.0 \pm 1.5$  S.D.  $\pm 7.4$  ( $n = 42$ ) before intravenous injection of 1.6 mg of lanatoside C and  $25.8 \pm 2.3$  S.D. 15 ( $n = 42$ ) after (table 2). Digitalization by lanatoside C thus brought a marked orthostatic reaction, the difference being highly significant ( $p < 0.001$ ).

A similar result was obtained with the lower doses of lanatoside C. Before digitalization the orthostatic reaction was  $16.3 \pm 3.2$ , S.D. 10. The corresponding values after digitalization were  $25.3 \pm 2.8$  S.D. 11.4. Again the difference is highly significant ( $p < 0.001$ ) ( $n = 17$ ).

### B. Digitoxin

The orthostatic pulse reaction before digitoxin was  $16.3 \pm 2$ , S.D.  $\pm 10$  ( $n = 23$ )

and after  $15.7 \pm 1.9$  beats/min, S.D.  $\pm 9.0$ . There is thus no difference before and after digitoxin per os ( $p = 0.6-0.5$ ).

### C. Digoxin

In the digoxin group the orthostatic pulse reaction before medication was  $12.9 \pm 1.7$  S.D.  $\pm 5.4$  beats/min and after a single oral dose of 1.25 mg  $14.6 \pm 3.5$  S.D. 11. Here again there is no difference ( $p = 0.6-0.5$ ).

## Comparison between different groups

Table 2 contains data of the entire material before digitalization and after different doses of lanatoside C and digitoxin.

At rest the lowest values of heart rate were obtained after digitoxin. The difference is fairly small, however, about 5 beats/min, though probably significant ( $p = 0.05-0.02$ ). The other drugs showed no significant difference compared with the predigitalization figures ( $p = 0.6-0.5$ ). Considering the heart rates in standing the lanatoside C groups differ from the others, i.e. the standing rate is higher about 90 beats/min, compared with 78 after digitoxin and 79 after digoxin. Before administration the heart rate was 83 beats/min.

The rise in heart rate after lanatoside C is probably significant ( $p = 0.05-0.01$ ) after the two other glucosides there was no significant difference in standing position. The orthostatic pulse reaction is most affected after lanatoside C, as had appeared earlier the difference is highly significant ( $p < 0.001$ ). There is undeniably significant difference in standing position. C in the standing position than to digitoxin and digoxin.

Table 2 Heart rate at rest 8 minutes standing and difference between rest and standing, in beats/min. ( $M \pm S.E., S.D.$ ) The effect of different glaucoma medicines.

		rest	standing	orthostatic pulse reaction
Before digitalization	69	$68 \pm 1.3$ 11	$83 \pm 1.7$ 14	$14.4 \pm 1.0$ 7.9
After lanatoside C, 1.6 mg	42	$63 \pm 2.0$ 13	$91 \pm 3.1$ 12	$25.8 \pm 2.3$ 15.1
After lanatoside C 0.4—0.8 mg	17	$63 \pm 2.0$ 8	$90 \pm 3.4$ 14	$23.3 \pm 2.8$ 11.4
After digoxin	23	$65 \pm 2.3$ 11	$78 \pm 5.0$ 14	$13.7 \pm 1.9$ 9.0
After digoxin	10	$65 \pm 3.3$ 11	$79 \pm 5.2$ 17	$14.6 \pm 3.5$ 11.0

Table 3 Heart rate at rest 8 minutes standing and difference between rest and standing, in beats/min. ( $M \pm S.E., S.D.$ ) Comparison between the effect of lanatoside C and digoxin on same subjects

		rest	standing	orthostatic pulse reaction
Before digitalization	14	$64 \pm 2.9$ 11	$79 \pm 3.7$ 13.8	$14.4 \pm 2.3$ 8.6
After lanatoside C, 1.6 mg	14	$63 \pm 4.2$ 16	$86 \pm 4.6$ 17	$22.3 \pm 3.6$ 13.5
After digoxin	14	$63 \pm 3.2$ 12	$80 \pm 4.0$ 13	$16.9 \pm 2.3$ 8.8

(women) before digoxin and, respectively 113 and 107 after. The differences are not significant ( $p = 0.6-0.4$  and  $0.2-0.1$ ).

Nor was there any difference 3 minutes after exercise between the values before and after administration of digoxin.

#### C. Digoxin

The same remarks apply to the heart rate reaction after exercise in conjunc-

tion with digoxin medication as with lanatoside C and digoxin.

Before digoxin the heart rate was  $107 \pm S.D. 16$  beats/min ( $n = 10$ ) immediately after exercise, after digoxin  $104 \pm S.D. 16$  the difference is not significant ( $p = 0.3-0.2$ ). Three minutes after exercise the figures were, respectively  $92 \pm S.D. 8$  and  $90 \pm S.D. 7$  again without significant difference. (In the digoxin group men and women are presented together)

At a load of 300 kpm/min the men's heart rate was 100 beats/min before and 96 after *digitoxin*. The difference is not significant ( $p = 0.2-0.1$ )

At 600 kpm/min the corresponding figures were 129 and 124 beats/min, again being non-significant ( $p = 0.1-0.05$ )

At 900 kpm/min the heart rate was 160 beats/min before and 153 beats/min, after *digitoxin*. The difference being probably significant ( $p = 0.02-0.01$ )

At the highest load of 1200 kpm/min the rates were respectively 169 and 167 beats/min, the difference being non-significant

For women the tendency was the same as for men. At 200 kpm/min the rate was 95 beats/min before and 92 after *digitoxin*, the difference is not significant ( $p = 0.02-0.1$ ). At 400 kpm/min the figures were 121 and 120 again no difference ( $p = 0.8-0.7$ ). At 600 kpm/min they were 156 and 154 ( $p = 0.5-0.4$ ) at 800 kpm/min 170 and 165 the difference being probably significant ( $p = 0.02-0.01$ ). Only six subjects, however exercised at this last load.

#### C. Digoxin

The subjects who were given *digoxin* per os were comparatively few altogether 10 of whom 6 men and 4 women. Since men and women exercised at different loads, they are recorded separately and the material is judged to be too small for statistical analysis.

At a load of 300 kpm/min the average heart rate for men before *digoxin* was 91 beats/min (range 66-106) and 90 after (range 68-118)

At 600 kpm/min the figures were 121 (range 90-136) before and 117 (range 92-152) after *digoxin*, at 900 kpm/min 149 (range 122-168) before and 142 (range 92-152) after

For women the rate at 200 kpm/min before *digoxin* was 96 beats/min (range 86-106) and 100 after (range 84-120). At 400 kpm/min the figures were 119 (range 110-130) before and 118 (range 100-136) after and at 600 kpm/min 155 (range 136-164) before and 150 (range 128-168) after *digoxin*.

The heart rate reaction during exercise showed the same tendency after *digoxin* per os as after *digitoxin*.

#### 4 Heart rate after exercise

The heart rate immediately and 3 minutes after the end of exercise is illustrated in tables 5 and 6

There was no difference before and after digitalization either with *lanatoside C*, *digitoxin* or *digoxin*. Men and women had the same pulse reaction after exercise.

##### A. Lanatoside C

The men's heart rate immediately after the end of exercise was 108 beats/min before and 109 after digitalization (table 5). The corresponding figures for women were 113 and 108 beats/min, the difference before and after digitalization being non-significant ( $p = 0.2-0.1$ ). Three minutes after the end of exercise the figures for nondigitalized subjects were 96 beats/min (men) and 94 beats/min (women) after digitalization 94 and 92. In neither case was the difference significant ( $p = 0.4-0.2$ )

##### B. Digitoxin

In the case of *digitoxin* as well the heart rate after exercise was little affected in relation to the situation before digitalization.

Immediately after exercise the heart rates were 111 beats/min (men) and 114

*Table 2. Heart rate at rest 3 minutes standing and difference between sit and stand 1 in beats/min. ( $M \pm S.E., S.D.$ ) The effect of different glucoside*

	n	rest	standing	orthostatic pulse reaction
Before digitalization	69	$68 \pm 1.3$ 11	$83 \pm 1.7$ 14	$14.4 \pm 1.0$ 7.9
After lanatoside C, 1.6 mg	42	$63 \pm 2.0$ 13	$91 \pm 3.1$ 12	$23.8 \pm 1.5$ 15.1
After lanatoside C 0.4-0.8 mg	17	$65 \pm 2.0$ 8	$90 \pm 3.4$ 14	$25.5 \pm 2.8$ 11.4
After digoxin	23	$63 \pm 2.5$ 11	$78 \pm 3.0$ 14	$15.7 \pm 1.9$ 9.0
After digoxin	10	$63 \pm 3.5$ 11	$79 \pm 3.2$ 17	$14.6 \pm 3.5$ 11.0

*Table 3. Heart rate at rest 3 minutes standing and difference between sit and standing in beats/min. ( $M \pm S.E., S.D.$ ) Comparison between the effect of lanatoside C and digoxin on same subject*

		rest	standing	orthostatic pulse reaction
Before digitalization	14	$64 \pm 2.9$ 11	$79 \pm 3.7$ 13.6	$14.4 \pm 2.3$ 8.6
After lanatoside C 1.6 mg	14	$63 \pm 4.2$ 16	$86 \pm 4.6$ 17	$22.5 \pm 3.6$ 13.3
After digoxin	14	$63 \pm 3.2$ 12	$80 \pm 4.0$ 13	$16.9 \pm 2.3$ 8.8

(women) before digoxin and, respectively 113 and 107 after. The differences are not significant ( $p = 0.6-0.4$  and  $0.2-0.3$ ).

There was no difference 3 minutes after exercise between the values before and after administration of digoxin.

#### C. Digoxin

The same remarks apply to the heart rate reaction after exercise in conjunc-

tion with digoxin medication as with lanatoside C and digoxin.

Before digoxin the heart rate was  $107 \pm S.D. 16$  beats/min ( $n = 10$ ) immediately after exercise, after digoxin  $104 \pm S.D. 16$  the difference is not significant ( $p = 0.3-0.2$ ). Three minutes after exercise the figures were, respectively  $92 \pm S.D. 8$  and  $90 \pm S.D. 7$  again without significant difference. (In the digoxin group men and women are presented together).

Table 4 Heart rate at rest 8 minutes standing and difference between rest and standing ( $M \pm S.E. S.D.$ ) in beats/min.

	n	rest	standing	orthostatic pulse reaction
Before lanatoside C no symptoms	30	$68 \pm 2.1$ 11	$81 \pm 2.6$ 15	$13.3 \pm 1.3$ 7
After lanatoside C no symptoms	30	$64 \pm 2.5$ 14	$87 \pm 3.5$ 19.2	$23.0 \pm 1.8$ 10
Before lanatoside C, symptoms	12	$71 \pm 3.4$ 17	$88 \pm 4.2$ 15	$17.5 \pm 2.2$ 8
After lanatoside C, symptoms	12	$66 \pm 3.4$ 12	$99 \pm 3.7$ 20	$32.9 \pm 3.2$ 12
Before digitoxin, no symptoms	10	$67 \pm 3.9$ 12	$77 \pm 3.9$ 12	$9.3 \pm 1.6$ 5
After digitoxin no symptoms	10	$67 \pm 3.7$ 12	$80 \pm 4.3$ 14	$12.7 \pm 1.9$ 6
Before digitoxin, symptoms	13	$66 \pm 2.4$ 9	$81 \pm 3.5$ 13	$15.5 \pm 1.9$ 7
After digitoxin, symptoms	13	$59 \pm 2.6$ 9	$77 \pm 4.3$ 15	$18.0 \pm 2.2$ 8

## Effect on certain aspects of circulatory function and dimensions

### 1 Working capacity at a heart rate of 170 beats/min (W<sub>170</sub>)

In view of the linear relationship between load and heart rate (Wahlund 1948) and the fact, as noted previously that the heart rate was almost identical at comparable loads before and after digitalization, it is natural that no appreciable difference in values of W<sub>170</sub> appeared as a result of the method of digitalization.

#### A. Lanatoside C

W<sub>170</sub> was, for men,  $1055 \pm 42$  kpm/min (S.D.  $\pm 192$ ) before and  $1038 \pm 41$  kpm/min (S.D.  $\pm 189$ ) after administra-

tion of lanatoside C intravenously ( $n = 21$ ). The difference is not significant ( $p = 0.2-0.1$ ). For women the figures were  $615 \pm 39$  kpm/min before and  $640 \pm 27$  after digitalization ( $n = 21$ ). Again there was no significant difference before and after lanatoside C ( $p = 0.1-0.05$ ).

#### B. Digitoxin

In this group the figures of W<sub>170</sub> for men were  $1087 \pm 69$  kpm/min (S.D.  $\pm 230$ ) before and  $1130 \pm 67$  (S.D.  $\pm 224$ ) after digitalization ( $n = 11$ ). The difference is not significant ( $p = 0.1-0.05$ ).

The corresponding figures for women in this group were  $695 \pm 40$  and  $714 \pm 36$  kpm/min, respectively ( $n = 12$ ). Again there was no significant difference before and after digitoxin ( $p = 0.2-0.1$ ).

Table 3 Heart rate (beats/min.) before and after administration of 1 ml id G  
( $M \pm S.E., S.D.$ )

Sex	Work load, kpm/min.	No. of subjects	Heart rate, beats/min.	
			before	after
M	resting	21	$66 \pm 2.3$ 11	$64 \pm 2.8$ 15
	standing	21	$79 \pm 2.9$ 15	$87 \pm 3.6$ 17
	300	21	$98 \pm 2.8$ 15	$99 \pm 3.3$ 15
	600	21	$126 \pm 3.2$ 15	$127 \pm 3.5$ 16
	900	21	$157 \pm 3.6$ 17	$157 \pm 3.6$ 17
	1200	12	$168 \pm 1.9$ 7	$168 \pm 2.5$ 8
	rested. after work	21	$108 \pm 3.0$ 14	$109 \pm 4.0$ 19
	3 min after work	21	$95 \pm 1.9$ 9	$96 \pm 2.6$ 11
F	resting	21	$72 \pm 2.5$ 12	$66 \pm 3.2$ 15
	standing	21	$87 \pm 3.6$ 16	$94 \pm 4.8$ 22
	200	21	$107 \pm 3.0$ 14	$108 \pm 3.6$ 16
	400	21	$134 \pm 3.9$ 18	$133 \pm 3.6$ 16
	600	18	$160 \pm 3.6$ 15	$160 \pm 3.3$ 14
	800	7	$170 \pm 3.9$ 10	$168 \pm 2.1$ 8
	rested after work	21	$113 \pm 4.0$ 19	$108 \pm 4.5$ 21
	3 min after work	21	$94 \pm 2.9$ 15	$94 \pm 3.4$ 16

Table 6 Heart rate (beats/min) before and after administration of digitoxin  
( $M \pm S.E., S.D$ )

Sex	Work load, kpm/min	No. of subjects	Heart rate, beats/min	
			before	after
M	resting	11	$67 \pm 2.9$ 10	$64 \pm 3.5$ 12
	standing	11	$81 \pm 2.9$ 10	$81 \pm 4.9$ 16
	300	11	$100 \pm 3.5$ 11	$96 \pm 3.8$ 12
	600	11	$129 \pm 4.5$ 15	$124 \pm 4.5$ 15
	900	11	$160 \pm 4.7$ 16	$153 \pm 5.1$ 17
	1200	6	$169 \pm 3.5$ 11	$167 \pm 3.8$ 9
	immed. after work	11	$111 \pm 4.7$ 16	$113 \pm 4.4$ 15
	3 min. after work	11	$96 \pm 2.6$ 9	$94 \pm 3.2$ 10
F	resting	12	$66 \pm 3.2$ 11	$62 \pm 3.0$ 10
	standing	12	$78 \pm 4.3$ 15	$76 \pm 3.7$ 13
	300	9	$95 \pm 4.6$ 14	$92 \pm 3.6$ 11
	400	11	$121 \pm 5.2$ 17	$120 \pm 4.5$ 15
	600	11	$156 \pm 4.7$ 15	$154 \pm 4.8$ 16
	800	6	$170 \pm 4.7$ 11	$165 \pm 4.3$ 11
	immed. after work	12	$114 \pm 4.4$ 15	$107 \pm 5.4$ 19
	3 min. after work	11	$94 \pm 3.4$ 11	$92 \pm 4.9$ 16

In this group the  $W_{170}$  for men was 1112 kpm/min (range 970–1315) before and 1074 (range 950–1320) after digitalization ( $n = 6$ )

The figures for women were 682 kpm/min (range 611–770) and 690 (range 620–760) respectively ( $n = 4$ ). The results are entirely in accordance with the findings for lanatoside C and digitoxin.

The occurrence of subjective symptoms, i.e. signs of digitalis intoxication, caused no change in  $W_{170}$ .

In the lanatoside C group four men had subjective symptoms their average  $W_{170}$  was 1003 kpm/min before digitali-

zation and 994 after. Eight women had subjective symptoms after lanatoside C their  $W_{170}$  was 619 before digitalization and 611 kpm/min after ( $p = 0.8-0.7$ )

In the digitoxin group six men had subjective symptoms their  $W_{170}$  before digitalis was 1196 kpm/min before and 1218 kpm/min after ( $p = 0.1-0.03$ ). For women in this group the figures were, respectively 649 and 673 kpm/min ( $n = 7$ ) ( $p = 0.3-0.2$ )

## 2. Total amount of haemoglobin

Total haemoglobin did not show any change after digitalization.

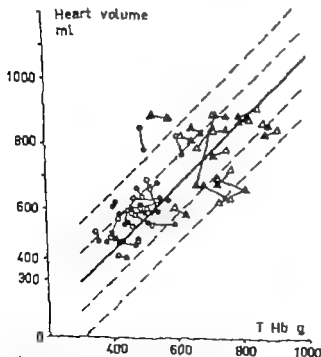


Fig. 3.

Heart volume in relation to total haemoglobin. The continuous line represents the normal regression line described by Holmgren et al. (1957). The dotted lines indicate  $\pm$  standard deviation.

Unfilled circles represent women before digitalization, filled circles after. Unfilled triangles represent men before digitalization, filled triangles after.



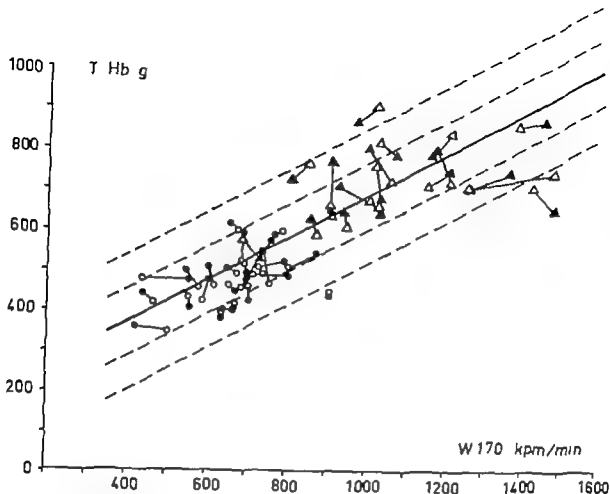


Fig 4 Total haemoglobin in relation to working capacity at pulse 170 Wirs. Normal regression line and symbols as in fig. 3

Before 1.6 mg of lanatoside C intravenously the total haemoglobin for that group of male subjects was  $712 \pm 20$  g (S.D.  $\pm 87$ ) and  $723 \pm 20$  g (S.D.  $\pm 82$ ) after ( $n = 19$ ). The difference is not significant ( $p = 0.6-0.5$ ). For women the figures were  $454 \pm 9$  and  $462 \pm 13$  g respectively ( $n = 21$ ) ( $p = 0.8-0.7$ ).

In the digitoxin group total haemoglobin was determined before and after medication in nine subjects, 2 men and 7 women. For the men the mean total haemoglobin was 800 g before and 845 g

after digitalization. For the women the means were 514 g (range 423-600) before and 431 g (range 423-590) after.

The haemoglobin concentration (g/l) was determined in all subjects before digitalization and in 13 after.

Before digitalization the concentration was  $13.2 \pm 0.34$  g/l S.D.  $\pm 1.2$  and  $13.8 \pm 0.26$  g/l after ( $p = 0.1-0.05$ ). The blood volume was in these cases  $4.5 \pm 0.25$  l before and  $4.3 \pm 0.21$  after. There was no significant difference ( $p = 0.5-0.4$ ).

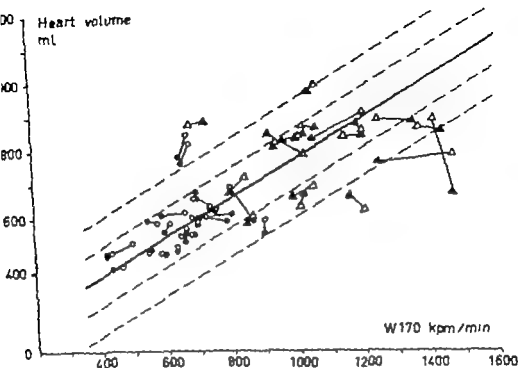


Fig. 5. Heart volume in relation to working capacity at pulse 170 W/min. Normal regression line and symbols as in fig. 3.

### 3 Heart volume in prone position

The heart volume in prone position was determined before digitalization in all subjects. After digitalization, prone heart volume determinations were for technical reasons made on 17 men and 18 women in the lanatoside C series and on 8 subjects (2 men and 6 women) in the digitoxin series.

The heart volume (in millilitres) was for men  $814 \pm 28$  ml (S.D.  $\pm 131$ ) before lanatoside C and  $807 \pm 25$  ml (S.D.  $\pm 102$ ) after ( $p = 17$ ). For women the figures were  $566 \pm 24$  ml (S.D.  $\pm 101$ ) before and  $537 \pm 21$  ml (S.D.  $\pm 82$ ) after ( $n = 18$ ).

For the digitoxin group the figures for

the 8 subjects were 669 (range 490–860) before and 642 (range 480–850) after.

There was no significant difference in prone heart volume either after lanatoside C or after digitoxin ( $p = 0.2-0.1$ ).

### 4 Heart volume in standing position

It is well known that the heart volume is larger in the prone than in the standing position (Nylin 1934; Larsson & Kjellberg 1948; Kjellberg et al. 1949; 1951; Sjöstrand 1956; Linderholm & Strandell 1958). This has been explained on the basis of the redistribution of the blood volume which reduces the heart (Björke & Laurell 1927; Jonell 1939; Jon-

Table 7 *Lenetoride C 1.6 mg Lp*

Sex	Nr of subj	Work load kpm/min.	Heart rate beats/min.		Ventilation l/min. BTPS	
			before	after	before	after
M	16	rest	65 ± 2.4 10	61 ± 2.8 11	7.8 ± 0.40 1.4	8.2 ± 0.47 1.6
	11	300	100 ± 3.3 11	99 ± 3.6 12	20.3 ± 0.58 2.0	21.4 ± 0.84 2.8
	12	600	128 ± 3.5 14	118 ± 4.5 16	32.0 ± 0.68 2.4	33.9 ± 0.83 2.9
	11	900	158 ± 5.1 17	157 ± 4.3 14	45.7 ± 1.86 6.1	47.5 ± 1.78 6.2
	8	1200	168 ± 2.5 7	163 ± 3.5 9	60.7 ± 4.05 11.3	62.9 ± 5.04 14.1
F	16	rest	79 ± 3.4 13	69 ± 4.1 16	7.6 ± 0.51 2.1	7.4 ± 0.58 2.3
	16	200	106 ± 3.8 15	106 ± 1.4 6	18.6 ± 0.98 3.9	19.4 ± 1.4 5.7
	13	400	128 ± 6.1 22	126 ± 4.6 17	27.0 ± 1.19 4.3	27.1 ± 1.21 4.4
	11	600	155 ± 4.9 16	152 ± 4.4 15	39.5 ± 1.71 5.6	41.4 ± 2.7 8.9
	4	800	165 ± 3.5 7	160 ± 4.5 9	55.7 ± 3.25 6.5	52.6 ± 4.1 8.1

Table 8 *Digitoxin*

Sex	Nr of subject	Work load kpm/min.	Heart rate, beats/min.		Ventilation, l/min. BTPS	
			before	after	before	after
F	8	rest	69 ± 2.9 8	67 ± 3.9 11	7.4 ± 0.36 1.02	6.1 ± 0.35 0.7
	7	200	96 ± 3.6 9	95 ± 3.5 9	17.3 ± 0.79 2.1	15.7 ± 0.64 1.7
	8	400	120 ± 4.0 11	122 ± 4.9 14	26.1 ± 1.14 3.2	24.2 ± 1.23 3.5
	6	600	155 ± 3.2 8	153 ± 4.1 10	38.1 ± 3.5 8.6	35.6 ± 1.3 3.2

Table 7 (continued)

Oxygen uptake ml/min. NTPD		Mechanical efficiency per cent		Lactate, mM/l	
before	after	before	after	before	after
259 ± 10.3 40	293 ± 10.6 40			1.23 ± 0.12 0.44	1.30 ± 0.06 0.52
864 ± 24 79	887 ± 26.7 88	25.4 ± 0.39 1.3	23.4 ± 0.83 2.8	1.64 ± 0.22 0.74	1.54 ± 0.09 0.52
1418 ± 17.5 60	1445 ± 21.8 76	24.2 ± 0.27 0.9	24.6 ± 0.58 2.0	2.30 ± 0.30 1.10	2.34 ± 0.23 0.92
1980 ± 33.4 110	2047 ± 22.5 74	24.9 ± 0.34 1.1	25.4 ± 0.44 1.5	4.40 ± 0.57 1.88	4.59 ± 0.59 1.95
2603 ± 67.9 190	2620 ± 61.1 171	24.4 ± 0.37 1.6	24.4 ± 0.64 1.8	4.51 ± 0.64 1.8	5.06 ± 0.89 2.5
224 ± 2.5 34	212 ± 10.3 41			1.42 ± 0.11 0.42	1.50 ± 0.12 0.46
709 ± 23.2 100	690 ± 26.3 105	19.5 ± 0.88 3.4	19.5 ± 0.86 3.4	1.61 ± 0.13 0.51	1.64 ± 0.18 0.71
1001 ± 21.9 79	998 ± 22.6 82	24.6 ± 0.43 1.7	24.0 ± 0.63 2.5	2.35 ± 0.25 0.84	2.16 ± 0.21 0.76
1383 ± 28.2 93	1408 ± 38.9 129	24.7 ± 0.50 1.8	23.9 ± 0.66 2.2	3.72 ± 0.39 1.29	3.76 ± 0.52 1.09
1812 ± 53.2 106	1771 ± 92 185	24.0 ± 0.60 1.2	24.7 ± 0.85 1.9	5.14 ± 0.48 0.95	4.95 ± 0.44 0.87

Table 8 (continued)

Oxygen uptake ml/min. NTPD		Mechanical efficiency per cent		Lactate, mM/l	
before	after	before	after	before	after
254 ± 14 40	218 ± 6 17			1.2 ± 0.08 0.23	1.4 ± 0.11 0.31
754 ± 35 93	667 ± 41 116	19.5 ± 1.01 2.7	20.4 ± 1.8 4.8	1.5 ± 0.06 0.16	1.5 ± 0.11 0.29
1021 ± 60 170	1025 ± 60 170	24.1 ± 1.2 2.9	24.2 ± 0.98 2.8	2.1 ± 0.35 1.0	2.1 ± 0.15 0.4
1336 ± 12 29	1391 ± 40 99	25.5 ± 0.63 1.4	24.7 ± 0.17 0.4	3.1 ± 0.41 1.0	4.0 ± 0.34 0.83

Table 9 *Mechanical efficiency*Comparison between 1) use of measured resting value, 2) use of basal  $O_2$  uptake according to Harris-Benedict.

Sex	Load	n		$\bar{x}$	$\bar{d}$	P
F	300	23	1	23.4	—1.4	<0.001
			2	22.0		
	600	26	1	23.2	—0.9	<0.001
			2	24.3		
	900	26	1	23.2	—0.6	<0.001
			2	24.6		
	1200	12	1	23.4	—0.3	<0.001
			2	24.9		
M	200	41	1	20.2	—1.1	<0.001
			2	19.1		
	400	41	1	24.9	—1.1	<0.001
			2	23.8		
	600	34	1	24.8	—1.1	<0.001
			2	23.7		
	800	8	1	24.7	—0.7	<0.001
			2	24.0		

sell & Sjöstrand 1941) The reduction of heart volume is accompanied by a change of pulse rate (Larsson & Kjellberg 1948) and stroke volume (Kjellberg et al 1951)

However there need not be any definite correlation between prone heart size and pulse rate (Linderholm & Strandell 1958). Since at the beginning of the lanatoxide C series of experiments, it was found that the standing heart rate increased after digitalis glucoside, the heart volume both in standing and prone positions was determined in 23 subjects before and after intravenous administration of 1.6 mg of lanatoxide C. Before administration it was  $640 \pm 32$  ml (S.D.  $\pm 153$ ) in the standing and  $693 \pm 32$  ml in the prone position. The difference between standing and

prone was highly significant ( $p < 0.001$ )

After lanatoxide C the standing heart volume was  $607 \pm 30$  ml (S.D.  $\pm 144$ ) and the prone  $685 \pm 32$ . Here again the difference between standing and prone was highly significant ( $p < 0.001$ ). Before digitalization the standing heart volume therefore was 640 ml, and after 607 ml. The difference in standing heart volume before and after lanatoxide C was probably significant ( $p = 0.05-0.02$ ) but this difference is on the other hand not striking when it is thought that the error of measurement was about 4%.

The conditions were similar in the digitoxin group. The standing heart volume was on an average  $619 \pm 49$  ml (S.D.  $\pm 146$ ) before and  $557 \pm 33$  ml (S.D.  $\pm$

150) after digitalization ( $n = 8$ ) Again the difference was probably significant ( $p = 0.05-0.02$ )

Before digitalization there was in my material no correlation between standing heart volume and standing heart rate ( $r = -0.33$   $p = 0.5-0.1$ ) After digitalization the correlation was probably significant ( $p = 0.05-0.02$ ) though still weak ( $r = -0.42$ )

### 5 Relationships between Different Circulatory Data and $W_{170}$

Mean values of  $W_{170}$ , prone heart volume and total haemoglobin have been reported in previous sections. Kjellberg et al. (1949) showed that there is a linear relationship between  $W_{170}$  on the one hand and prone heart volume and total haemoglobin on the other

In figs. 3, 4 and 5 the individual values before and after digitalization are plotted in relation to the normal regression line.

### 6 Effect on Oxygen Consumption, Mechanical Efficiency and Lactate Concentration

The values for heart rate, oxygen consumption, mechanical efficiency and lactate concentration are summarized in tables 7 and II. From tables 7 and 8 it will be seen that  $V_{O_2}$  per work load is roughly at the same level as found by P. O. Astrand (1935) and I. Astrand (1960) for submaximal exercise. After digitalization no appreciable change occurs either with lanatoside C or digitoxin. The same applies to the lactate concentration.

On the other hand the values for mechanical efficiency were throughout higher in my material than those of P. O. Astrand (1932) and I. Astrand (1960)

This is probably because I subtracted the measured resting value for  $V_{O_2}$ , while P. O. and I. Astrand used a value predicted as "basal" from tables. For the sake of comparison I have calculated the mechanical efficiency on a larger material in both ways, i.e. using either the measured resting value or a value predicted from Harris-Benedict's tables.

From table 9 it will be seen that there is a systematic difference between the two sets of values, the mechanical efficiency being constantly lower when predicted from tables. The difference is highly significant. It is then found that, using the same bases in calculation as I and P. O. Astrand, my figures of mechanical efficiency at least as far as women are concerned, are the same as found by I. Astrand in 1960. For men my values are rather higher than reported by P. O. Astrand in 1932. There is no difference in mechanical efficiency before and after cardioactive glycosides ( $p > 0.1$ ) (fig. 6)

### 7 Effect on Blood Pressure

There was no effect on either the systolic or diastolic blood pressure (measured by the cuff method) at rest or standing after injection of lanatoside C. The systolic blood pressure before digitalization was  $121 \pm 2.5$  (S.D. 12,  $n = 22$ ) and the diastolic  $78 \pm 2.0$  (S.D. 5.2). The corresponding values after digitalization were  $120 \pm 2.8$  (S.D. 12.4) and  $76 \pm 2.2$  (S.D. 10). In standing position the systolic blood pressure before digitalization was  $119 \pm 3.7$  and the diastolic  $79 \pm 2.1$ . After digitalization the corresponding values were  $117 \pm 2.6$  and  $75 \pm 2.2$ . The differences in blood pressures before and after digitalization are not significant ( $p = 0.3-0.2$ )

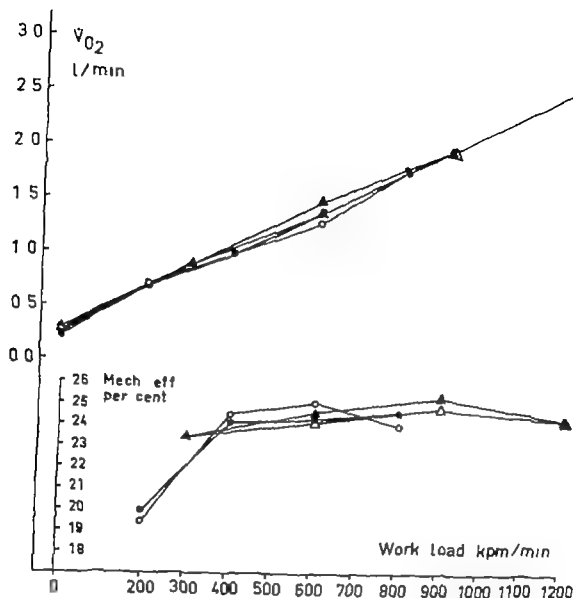


Fig. 6. Oxygen uptake and mechanical efficiency in relation to work load before and after digitalization. (Open symbols = before and filled symbols after)

## Discussion

### Subjective symptoms

Are subjective symptoms of any kind a sign of toxicity and by definition thus also of full digitalization or are e.g. gastrointestinal symptoms after oral doses a sign of local irritation and therefore no definite evidence of digitalization?

The galenicals are generally considered to have an irritative effect on the gastro-

intestinal tract. Gold (1942) compared single doses of *folium digitalis* with, as he considered, equivalent doses of digitoxin and found a considerably higher percentage of subjective symptoms in the *folium* group. If nausea occurs after parenteral administration, it is ascribed to systemic toxicity. Opinions are divided, however, concerning oral medication. Gold et al. (1952) after clinical experiments in man drew the conclusion that vomiting after

oral administration of certain cardioactive glucosides is due chiefly or perhaps solely to a local gastrointestinal irritation since after oral administration of digitalis, vomiting does not necessarily coincide with other signs of toxicity and, above all, because the quantities presumed to be absorbed were sub-emetic on parenteral administration. The doses used by Gold et al. varied fairly considerably and can hardly be called equivalent. Moreover the experiments were made on cardiac patients in different degrees of incompensation. Earlier Gold et al. (1930) had shown that there is a latent period of 8–10 hours (for the last dose of digitalis before the onset of the emetic effect. The latent period for digitoxin was stated to be  $6\frac{1}{2}$ –21 hours and for digoxin 1–3 hours (Gold et al. 1932).

Hatcher et al. (1922) reported a central effect of digitalis. The same conclusion was reached by Borison & Wang (1951) and Borison (1952) who, in experiments on cats, showed that digitalis has both a central effect localized to the posttruncus area of the medulla, which they called the emetic trigger zone, and a peripheral effect which is not gastrointestinal but whose exact nature it has not yet been possible to determine. According to these authors the intestine plays a very small role or none at all, in digitalis vomiting. They speak both of early vomiting, which follows on an intravenous injection and is always arranged by a trigger zone operation, being thus of central origin, and of late vomiting which is neither of central nor gastrointestinal origin.

In Gold's experiments the period of latency was relatively long before symptoms appeared after oral administration of digitoxin. As appears from table 1 B, the period of latency after oral digitoxin in any experiments was also fairly long, between 5 and 12 hours, except in our case when

it was 3 hours after the last dose. So long a latent period does not tally with the symptoms being due solely to a local gastrointestinal effect.

But the situation appears to be different as regards the subjects who had nausea after a large single dose of digitoxin. In these cases the latent period for nausea was short, 40 minutes to 2 hours generally 1 hour (table 1 C) and the effect rapidly disappeared. Here the effect may be solely local. Gold et al. (1932) found roughly the same latent period for digitoxin. Nausea after intravenous lanatide C cannot reasonably be of gastrointestinal origin.

As regards the present investigations, it may be said in conclusion that subjective symptoms after digitoxin and lanatide C may be regarded as at least partly a general effect and by definition as a sign of full digitalization whereas this probably should not apply to the digitoxin group since their subjective symptoms followed so quickly after digitalization. The degree and nature of the symptoms were in most cases relatively mild, which conforms with earlier reports in the literature of the effect of digitalis in healthy persons. The symptoms are not so grave or lengthy as reported in clinical materials, although the doses used in the present study were of the same order of magnitude as in current clinical use. It is probable, therefore, that the origin and severity of the symptoms are dependent to a great extent on the clinical status of the patients.

#### Heart rate at rest

Usually the resting heart rate falls to a varying extent after digitalization of healthy subjects (Routter & Puddu 1935; Larsen & Nielsen 1936; Nielsen et al. 1939; Erickson & Fahr 1945; Koefung 1950; Yu et al. 1952; Seltzer et al. 1959). The effect disappears fairly quickly (e.g.



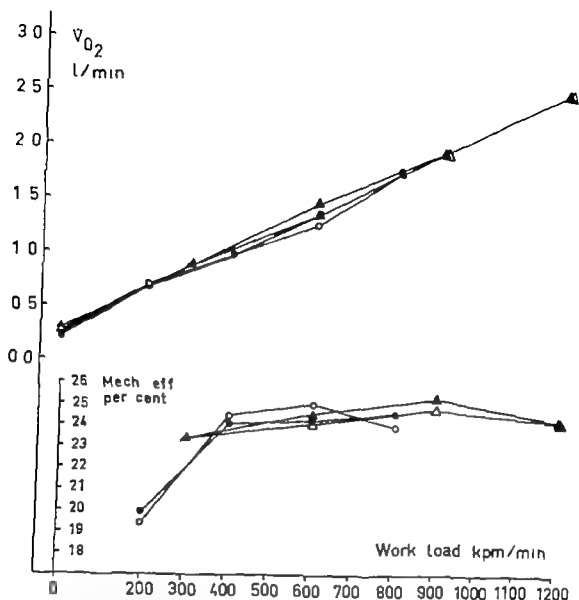


Fig. 6. Oxygen uptake and mechanical efficiency in relation to work load before and after digitalization. (Open symbols = before and filled symbols after)

## Discussion

### Subjective symptoms

Are subjective symptoms of any kind a sign of toxicity and by definition thus also of full digitalization or are e.g. gastrointestinal symptoms after oral doses a sign of local irritation and therefore no definite evidence of digitalization?

The galeicals are generally considered to have an irritative effect on the gastro-

intestinal tract. Gold (1912) compared single doses of folium digitalis with, as he considered, equivalent doses of digitoxin and found a considerably higher percentage of subjective symptoms in the folium group. If nausea occurs after parenteral administration it is ascribed to systemic toxicity. Opinions are divided, however concerning oral medication. Gold et al. (1952) after clinical experiments in man, drew the conclusion that vomiting after

creased highly significantly. This orthostatic reaction is still more pronounced in the subjects in the lanatoside C group who had subjective symptoms. The remarkable thing is that the digitoxin group did not react in this way: the standing heart rate was not changed by digitoxin, nor the orthostatic pulse reaction either. This is especially evident from table 3 which shows the results for those who received both lanatoside C and digitoxin. Nor did this increased orthostatic pulse reaction occur after digoxin. In respect of the orthostatic pulse reaction the lanatoside C, digitoxin and digoxin groups did not differ from one another prior to digitalization. The subjects represent normal material, and the standing heart rate and orthostatic pulse reaction were comparable with the findings in other normal materials composed of individuals with varying degrees of training (Holmgren et al. 1957).

It is possible that the difference in orthostatic reaction after lanatoside C, digitoxin and digoxin is caused by differences in the conditions of the tests in the present study.

Lanatoside C was injected. This may have created anxiety in the subjects, with consequent cardio-acceleration. There was, however, no difference in heart rate between the anxious series. Nor did the  $O_2$  uptake differ before and after lanatoside C. And, furthermore, the orthostatic test was not made until 2–3 hours after the lanatoside C injection and the subjects were accustomed to the experimental routine.

Another explanation of the orthostatic reaction induced by lanatoside C is to be sought in a central nervous effect of the drug; the effect of vagal tone may be accompanied by a decreased sympathetic tone, i.e. to peripheral resistance and capacity of vessels. Through a compensatory mechanism this would give rise in the standing position to a high sympathetic

tone with a marked increase of the heart rate in consequence. An effect on the peripheral mechanisms of vascular control cannot be ruled out, however.

The reaction in my experiments was greatest in the lanatoside C group with subjective symptoms, that is in the subjects in whom the digitalis effect was most definitely documented, and may be explained as above. I have observed a similar orthostatic reaction in a patient who had taken 15 g solium digitalis tablets for suicidal purposes. She was gravely intoxicated and had A-V block II with a heart rate of 35 beats/min. Her standing heart rate, on the other hand, was 95 beats/min. without A-V-block. It is difficult, however, to explain the difference in reaction between the lanatoside C group and the digitoxin group.

#### *Effect on Circulatory Adjustment during Exercise*

During work of low intensity the heart rate after digitalization was slightly lower than before. This is in agreement with the findings of Yu et al. (1952) and Williams et al. (1958) who used low loads throughout. At a higher working intensity on the other hand, there was no significant difference in heart rate, ventilation, oxygen uptake or lactate concentration during exercise with or without digitalis. Thus digitalis did not affect the calculated physical working capacity at maximal heart rate nor the working capacity at heart rate 170.

In general it may be said that — apart from the effect at low loads — digitalis does not change the circulatory response to exercise as regards the magnitude of the stroke volume, the increase of heart rate and cardiac output, the adjustment of the peripheral circulation or the mechanical efficiency.

Seltzer et al 1959 Koefbing 1950) The degree of lowering in relation to the pre-medication situation is not particularly great, however Yu et al (1952) found the reduction to be significant at the 1 per cent level. Seltzer et al. (1959) found that the heart rate admittedly fell considerably in individual cases but that the mean change in the group was not significant. The circumstances were similar in the present study. A few subjects had a greatly reduced heart rate, others a somewhat higher rate, and in some it was unchanged. The mean change for the entire material was comparatively insignificant. In this respect a difference appears to exist between those who had subjective symptoms after digitalis (both lanatoxide C and digitoxin) and those who remained free from symptoms. In the former group the reduction of heart rate after digitalization was probably significant. In those without symptoms there was no significant difference. It is possible that if all subjects had been in the same and fairly pronounced state of nausea, i.e. of fairly pronounced intoxication the effect might have been more uniform. But in experiments of this type it is hardly defensible to drive the degree of digitalization too far in healthy persons.

### *Vagal effect*

The digitalis glucosides have been said to have a potentiating influence on the effect of vagal activity to which is ascribed the reduced heart rate (Eichna et al. 1943 and others). Heymans & Heymans (1926) showed in cross-circulation experiments that digitalis does not have a direct effect on the vagal centres. They considered the primary bradycardia after injection of digitalis to derive from a reflex mechanism. Gremels (1935) considered that small quantities of strophanthin provoked an in-

creased irritability of the vagus nerve, while Abdon & Nielsen (1938) thought that the cardioinhibitory effect of digitalis on the vagal nerve was probably due solely to sensitization of the heart muscle to normal vagal tone. Later on, the question of the vagal effect of digitalis was reformulated by Abdon et al (1938) who maintained that effect on the myocardium was instead due to sensitization to acetylcholine.

Gold et al. (1939) after experiments on patients with atrial fibrillation before and after digitalis, thought the slower heart rate to be due to a combination of two factors: a vagal factor which is abolished by atropine and exercise, and an extravagal factor which is unaffected by atropine, and that the vagal factor generally predominates after moderate doses, the extravagal after large doses.

Discussion of the vagal effect has now fallen into the background. There is no doubt that digitalis does have a vagal effect, but this cannot be isolated from its relation to the haemodynamics as a whole and in itself probably plays a comparatively minor role.

### *The orthostatic pulse reaction*

The orthostatic pulse reaction, that is the difference between resting and standing heart rate, increased greatly after intravenous injection of lanatoxide C, both after small doses and after 1.6 mg. This increased pulse reaction might conceivably be due to the lower resting pulse rate and therefore be merely an apparent increase. As already noted the resting heart rate was admittedly rather lower but not significantly so. On the other hand the standing heart rate was higher after lanatoxide C both in small and large doses, the rise being probably significant ( $p = 0.02-0.01$ ). The orthostatic pulse reaction in

## RESULTS II

### Electrocardiographic Changes in conjunction with Digitalization

#### Methods of measurement

##### P-Q and Q-T

The measurements of the duration of P-Q (or P-R) and Q-T (or R-T) were made within the same R-R interval as was used for determination of the duration of R-R. The unit of measurement was 0.01 second and the durations are indicated as means of at least three measurements. In most cases P-Q and Q-T were measured in lead II and CR2 or CH2. CR2/CH2 were chosen since ST-T changes due to digitalization proved to be least pronounced in these leads.

The Q-T measurements have been difficult to perform, especially on electrocardiograms taken during exercise at high frequencies on which it was often impossible to decide where the T wave ends, an experience which is shared by all who have had to do with this problem (*inter alia* Grewin 1948, Lepeschkin & Surawicz 1952, Cannon & Sjöstrand 1953, Bengtsson 1956) the percentage of rejects has consequently been fairly high.

Q-T is not indicated in absolute figures but has been corrected for the frequency and is reported as an index "T" taken from nomogram presented by Goldberger (1955).

The ventricular gradient G and the mean QRS axis were measured by the method described by Ashman (1943) Ashman, Gardberg & Byer (1943) Ashman & Byer (1943) and Gardberg (1957).

The direction is indicated in degrees, the

size in units (one unit is equal to 4 microvolt seconds)

##### ST-T

The isoelectric line has been drawn between two P-Q junctions and the ST segment has been evaluated in relation to this isoelectric line. The ST segment was measured at the junction and depressions have been measured in units of 0.5 mm (1 mm = 0.1 mV).

The T waves were measured in units of 0.5 mm from the isoelectric line and their heights at rest and after exercise were statistically analysed. The T wave was also evaluated in conjunction with the ST segment, and the negative phase of the T wave has been calculated from the isoelectric line. For ST-T all leads were separately evaluated.

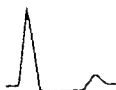
The importance of not only measuring ST-T depressions in absolute terms but also of describing the appearance and course of the ST-T segment has often been emphasized (Bengtsson 1957, Sandberg 1961, Lloyd-Thomsen 1961). When ST-T is measured and reported in absolute terms, as done, for example, by Grewin (1948) and Bengtsson (1956, 1957) the results can be statistically analysed, which is necessary for establishing normal variations. One loses the description of form, however and at the same time the overall evaluation of the ST-T region. All types of ST-T changes observed have earlier been described on the basis of measurements and analysis of the ST-T changes that appear in healthy subjects after digitalization (Nordström-Ohrberg 1959). This description has been used in the pre-

It also appears from these investigations that the degree of orthostatism in the standing position does not influence the circulatory response during sitting exercise. This is corroborated by the fact that the increase of heart rate during exercise after lanatoside C did not differ significantly from that after other digitalis drugs which produced no marked orthostatic reaction.

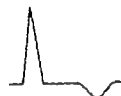
The lactate concentrations during exercise showed large intra and interindividual variations, as earlier found in untrained individuals (Holmgren & Ström 1959 I Astrand 1960). The relatively high lactate concentrations showed that exercise at the highest loads introduced a

fairly large anaerobic component into the total muscular metabolism, but that the exercise was not maximal. The lactate concentration values at the end of exercise were within the same order of magnitude before and during digitalization, indicating that the medication did not influence the ratio between aerobic and anaerobic metabolic components during exercise.

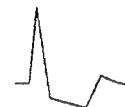
In conclusion, these studies have shown that the digitalis glucosides do not provoke such a haemodynamic effect on healthy subjects as to influence the circulatory response to an exercise test at submaximal loads to any important degree.



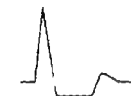
ST segment horizontally depressed  $>0.1$  mV —  $\geq 0.2$  mV  
T wave biphasic, the negative component  $\approx 0.2$  mV



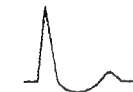
T wave negative  $>0.1$  mV  $\leq 0.4$  mV



ST segment obliquely downward sloping  $>0.2$  mV  $\leq 0.4$  mV  
T wave biphasic, the negative component  $\geq 0.4$  mV



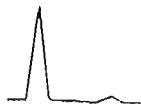
ST segment horizontally depressed  $0.3-0.4$  mV T wave biphasic, the negative component  $0.3-0.4$  mV



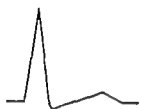
ST segment concave, sagging, what is called in German "mitdenformig". Inconsiderable or no depression at junction, the depression at its maximum in the middle  $0.1-0.2$  mV

## TYPES OF ST T CHANGES

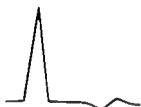
(Fig 7)



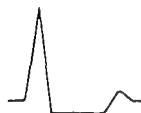
T wave  $\leq 0.1$  mV



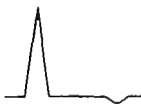
ST depressed =  $0.1$  mV with normal configuration, T wave positive  $\geq 0.1$  mV



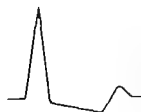
T wave biphasic (the negative component  $\leq 0.1$  mV)



ST segment horizontally depressed  $\leq 0.1$  mV T wave biphasic



T wave negative  $\leq 0.1$  mV



ST segment depressed  $0.1$  mV at junction, obliquely downward sloping, T wave biphasic, the negative component of the T wave  $> 0.1$  mV but  $< 0.3$  mV

beats/min after digitalization her heart rate was on an average 72 beats/min. She had also relatively pronounced subjective symptoms.

### C. Digoxin

The P-Q interval for the entire material prior to digoxin was on an average 14.0 msec, after digoxin 13.1 msec (table 11). The difference was not significant ( $p = 0.1-0.05$ ). No A-V block occurred.

## 2. Standing

### A. Lanatoside C

Before digitalization with 1.6 mg lanatoside C the P-Q interval in standing position was 14.7 msec for men and 13.8 msec for women. After digitalization the figures were 13.1 and 14.8 msec. The P-Q interval was thus, on an average, rather longer while, as previously noted, the heart rate was higher. The increase of the P-Q interval was slight, however and not significant ( $p = 0.1-0.05$ ). No subject had A-V block in standing position. The subject (no. 42) with prolonged P-Q interval at rest had a standing P-Q interval of 19 msec.

In the 0.4-0.8 mg groups the P-Q intervals before digitalization were  $15.1 \pm 2.4$  msec, after 0.4 mg  $15.3 \pm 2.1$  msec and after 0.8 mg  $15.3 \pm 2.1$  in fact entirely similar values.

### B. Digoxin

The P-Q interval before digoxin (table 10) was on an average 14.2 msec, and 13.8 msec after. The increase is not significant ( $p = 0.1-0.05$ ).

One subject (no. 47) who at rest after digoxin had A-V block II with P-Q interval up to 46 msec had a standing P-Q interval of 35 msec with heart rate of 96 beats/min.

### C. Digoxin

The standing P-Q interval before digoxin (table 11) was 13.8 msec and 13.6 msec after ( $p = 0.7-0.6$ ).

## 3. During exercise

### A. Lanatoside C

During exercise at 900 kpm/min the P-Q interval for men was the same before and after digitalization (12.6 msec) and for women at 600 kpm/min 11.8 msec before and 12.3 msec after. The difference is not significant ( $p = 0.2-0.1$ ).

### B. Digoxin

The P-Q interval was 11.6 msec before digoxin and 11.7 msec after (table 10). Even the subject (no. 47) who at rest after digitalization had a P-Q interval of 46 msec had exactly the same P-Q interval during exercise before and after digitalization.

### C. Digoxin

The P-Q interval did not change either after digoxin, as seen from table 11.

## 4. After exercise

### A. Lanatoside C

Immediately after exercise the mean predigitalization P-Q interval for men was 14.4 msec, and after 1.6 mg lanatoside C 15.1 msec. The difference, however is only probably significant ( $p = 0.05-0.02$ ). The heart rate was, however lower in the measured R-R interval after digitalization (table 9). For women, on the other hand, the P-Q interval after lanatoside C was 15.9 msec against 14.0 msec before, which is a significant difference ( $p = 0.01-0.001$ ). The heart rate, however is rather higher in the measured R-R interval after digitalization. One subject (no. 21) had a P-Q interval up to 26 msec after digitalization.



sent study with minor modifications (fig 7)

### Effect of cardioactive glucosides on rhythm and conduction

The results in respect of heart rate have been reported in an earlier section

#### *Effect on the rhythm*

At rest sinus arrhythmia and magnification of earlier sinus arrhythmia were common. In table 12 is shown the total incidence of sinus arrhythmia arising after digitalization (about 30 per cent of the cases) which percentually was fairly evenly divided among the various glucoside groups. In standing position naturally enough, the incidence was very much lower occurring in only 7 digitalized subjects.

During exercise the arrhythmia disappeared entirely except in one subject at the lightest load. Immediately after exercise it reappeared but diminished again 3 minutes after exercise, being then recorded in only 9 cases.

Sino-atrial block was recorded in one subject immediately after exercise

The frequency of premature beats (atrial and ventricular premature beats) was extremely low. One subject had ventricular premature beats resting standing, and during and after exercise, even before digitalization. In this person digitalization produced no increase in the number of premature beats. In other subjects occasional ventricular premature beats were recorded before digitalization.

After digitalization only occasional premature beats were recorded at rest, during and after exercise. At heavy loads no premature beats were recorded at all (table 12)

### *A V conduction*

#### *1 At rest*

##### *A Lanatoside C*

Intravenous injection of 1.6 mg of lanatoside C produced a brief prolongation of the P-Q interval at rest both in men and women (table 9) from 15.3 to 15.9 msec for men and from 14.7 to 15.4 msec in women. The prolongation was thus slight, yet probably significant ( $p = 0.05-0.02$ ).

In one case (no. 42) a conduction time of 19 msec at a heart rate of 76 beats/min was recorded before digitalization and 23 msec at 95 beats/min after lanatoside C.

In lower doses (0.4-0.8 mg) on the other hand lanatoside C produced no significant change in conduction time. Thus the P-Q interval before digitalization was on an average 16.3 msec ( $n = 9$ ) after 0.4 mg 16.8 and after 0.8 mg 18.3 ( $p = 0.1-0.05$ ). The greater prolongation of the P-Q interval after 0.8 mg lanatoside C is due to a very considerable prolongation in a 17 year-old subject (no. 63). The predigitalization conduction time of this person was 21 msec at 68 beats/min, and after 0.8 mg his heart rate was 72 beats/min and P-Q interval 35 msec. The P-Q interval of this subject was unaffected by 0.4 mg lanatoside C.

#### *II Digtoxin*

The atrioventricular conduction was generally little affected by digtoxin. Before administration the P-Q interval (table 10) was 14.8 msec, and 16.6 msec after. The difference is not significant ( $p = 0.2-0.1$ ). One subject (no. 47) had a greatly prolonged P-Q interval (up to 46 msec) with dropped ventricular complex, A V block II (7.6 6.5 5.4 block with Wenckebach periods). This subject (age 48 years) had a P-Q interval before digitalization of 19 msec at a heart rate of 60

P-Q, sec		I"	
before	after	before	after
$15.3 \pm 0.3$ 1.6 - 21	$15.9 \pm 0.4$ 1.9 - 21	$0.93 \pm 0.012$ 0.06 n = 21	$0.90 \pm 0.014$ 0.06
$14.7 \pm 0.4$ 1.6 - 20	$15.1 \pm 0.4$ 1.9 - 20	$0.93 \pm 0.015$ 0.07 n = 21	$0.96 \pm 0.017$ 0.08
$12.6 \pm 0.3$ 1.3 - 15	$12.6 \pm 0.4$ 1.3 - 15	$0.96 \pm 0.010$ 0.04 - 16	$0.97 \pm 0.009$ 0.02
$14.4 \pm 0.4$ 1.3 - 19	$15.1 \pm 0.5$ 2.0 - 19	$0.96 \pm 0.014$ 0.06 n = 19	$0.93 \pm 0.021$ 0.09
$15.6 \pm 0.4$ 1.8 - 21	$17.0 \pm 0.8$ 3.6 - 21	$1.00 \pm 0.015$ 0.07 - 21	$0.99 \pm 0.012$ 0.06
$14.7 \pm 0.5$ 2.4 - 20	$15.4 \pm 0.7$ 2.9 - 20	$0.98 \pm 0.017$ 0.07 n = 20	$0.92 \pm 0.018$ 0.08
$13.8 \pm 0.5$ 2.1 19	$14.8 \pm 0.6$ 2.7 19	$0.95 \pm 0.05$ 0.2 - 19	$0.98 \pm 0.02$ 0.07
$11.8 \pm 0.4$ 1.3 13	$12.3 \pm 0.4$ 1.3 13	$0.99 \pm 0.016$ 0.06 - 12	$0.99 \pm 0.016$ 0.06
$14.0 \pm 0.5$ 1.96 - 15	$15.9 \pm 0.7$ 2.8 - 15	$0.93 \pm 0.014$ 0.05 - 12	$0.94 \pm 0.03$ 0.12
$15.0 \pm 0.5$ 1 - 20	$16.2 \pm 0.8$ 3.6 - 20	$1.03 \pm 0.013$ 0.06 - 20	$1.02 \pm 0.011$ 0.05

Table 9  
P Q and R R intervals in  
csec (1/100 sec) and Q T  
interval expressed as index  
"I" before and after ad-  
ministration of 16 mg La-  
natosid C intravenously  
(M  $\pm$  S.E., S.D.)

Sex		R R, csec	
		before	after
Males	resting	95.6 $\pm$ 3.7 16.8 n = 21	99.9 $\pm$ 4.1 18.7
	standing	81.7 $\pm$ 2.6 11.7 n = 21	74.4 $\pm$ 3.2 14.5
	work 900 kpm/min.	37.8 $\pm$ 2.5 10.4 n = 18	39.4 $\pm$ 1.0 4.2
	immediately after work	53.7 $\pm$ 1.5 6.6 n = 21	57.4 $\pm$ 2.7 12.0
	3 min. after work	64.4 $\pm$ 1.4 6.6 n = 21	67.5 $\pm$ 1.4 6.3
Females	resting	83.7 $\pm$ 2.8 12.7 n = 20	93.0 $\pm$ 3.2 23.2
	standing	76.4 $\pm$ 4.4 14.8 n = 19	68.5 $\pm$ 4.5 19.1
	work 600 kpm/min.	37.7 $\pm$ 0.9 3.7 n = 16	37.9 $\pm$ 1.0 4.0
	immediately after work	57.5 $\pm$ 3.2 12.2 n = 14	56.4 $\pm$ 3.1 11.6
	3 min. after work	64.3 $\pm$ 2.2 10.0 n = 20	63.1 $\pm$ 2.5 11.2

P-Q, msec		T	
before	after	before	after
14.8 ± 0.5 23 ( = 23)	16.6 ± 1.6 7.0 ( = 23)	0.94 ± 0.014 0.063 ( = 23)	0.86 ± 0.013 0.060 ( = 19)
14.2 ± 0.5 2 ( = 22)	15.8 ± 1.2 5.3 ( = 20)	0.93 ± 0.015 0.072 ( = 23)	0.91 ± 0.012 0.052 ( = 19)
11.6 ± 0.3 11 18	11.7 ± 0.3 12 ( = 18)	0.95 ± 0.019 0.067 ( = 12)	0.93 ± 0.012 0.039 ( = 10)
14.6 ± 0.36 26 20	16.1 ± 1.34 6.79 = 19)	0.95 ± 0.016 0.069 ( = 19)	0.91 ± 0.012 0.049 (n = 18)
15.1 ± 0.48 29 = 23	17.1 ± 1.40 6.12 19)	0.99 ± 0.013 0.073 ( = 23)	0.94 ± 0.014 0.059 = 19)

P-Q, msec		T	
before	after	before	after
14.0 ± 0.6 19 10	13.1 ± 0.3 17 10)	0.90 ± 0.02 0.07 ( = 10)	0.89 ± 0.02 0.07 ( = 10)
13.8 ± 0.4 12 10	13.6 ± 0.6 20 10)	0.95 ± 0.02 0.03 = 10)	0.94 ± 0.01 0.04 ( = 10)
12.2 ± 0.3 8 6	11.7 ± 0.2 0.3 6)	0.97 ± 0.03 0.07 = 6)	0.97 ± 0.03 0.08 = 6
14.0 ± 0.3 10 10	13.9 ± 0.3 16 10	0.91 ± 0.01 0.04 ( = 10)	0.92 ± 0.02 0.03 = 10)
14.8 ± 0.6 18 10	15.1 ± 0.3 16 10	0.98 ± 0.02 0.03 10)	0.96 ± 0.02 0.06 ( = 10)

Table 10

P-Q and R R intervals in csec (1/100 sec) and Q T interval expr used as index I" before and after administration of digitoxin ( $M \pm S.E.$ , S.D.)

	R R, csec	
	before	after
resting	$91.5 \pm 3.0$ 14.4 (n = 23)	$99.5 \pm 3.3$ 14.7 (n = 23)
standing	$77.9 \pm 3.2$ 15.3 (n = 23)	$73.0 \pm 2.9$ 13.0 (n = 23)
during work	$36.6 \pm 0.7$ 2.9 (n = 16)	$37.1 \pm 0.7$ 2.4 (n = 16)
immediately after work	$55.6 \pm 1.9$ 8.7 (n = 20)	$61.4 \pm 3.1$ 13.9 (n = 20)
3 min. after work	$64.5 \pm 1.57$ 7.55 (n = 23)	$68.2 \pm 2.57$ 11.21 (n = 23)

Table 11

P-Q and R R intervals in csec (1/100 sec) and Q T interval expressed as dex I before and after administration of digoxin ( $M \pm S.E.$ , S.D.)

	R R, csec	
	before	after
resting	$98.4 \pm 4.8$ 15.3 (n = 10)	$100.5 \pm 11.1$ 19.3 (n = 10)
standing	$83.7 \pm 5.5$ 17.5 (n = 10)	$77.5 \pm 5.1$ 16.1 (n = 10)
during work	$40.3 \pm 1.3$ 3.1 (n = 6)	$39.2 \pm 0.9$ 2.3 (n = 6)
immediately after work	$60.9 \pm 4.1$ 13.1 (n = 10)	$60.4 \pm 3.8$ 12.2 (n = 10)
3 min after work	$68.3 \pm 3.0$ 9.4 (n = 10)	$71.0 \pm 3.4$ 10.8 (n = 10)

P-Q conc		I	
before	after	before	after
14.8 ± 0.5 2.3 ( = 23)	16.6 ± 1.6 7.0 ( = 23)	0.94 ± 0.014 0.063 ( = 23)	0.86 ± 0.013 0.060 ( = 19)
14.2 ± 0.5 1.1 ( = 22)	15.6 ± 1.2 5.3 ( = 20)	0.93 ± 0.015 0.072 ( = 23)	0.91 ± 0.012 0.052 ( = 19)
11.6 ± 0.3 1.1 ( = 10)	11.7 ± 0.3 1.2 ( = 18)	0.93 ± 0.019 0.067 ( = 12)	0.93 ± 0.012 0.039 (n = 10)
14.6 ± 0.58 2.61 ( = 20)	16.1 ± 1.54 6.73 ( = 19)	0.95 ± 0.016 0.069 ( = 19)	0.91 ± 0.012 0.049 ( = 18)
15.1 ± 0.48 2.9 ( = 23)	17.1 ± 1.40 8.12 ( = 19)	0.99 ± 0.015 0.073 ( = 23)	0.94 ± 0.014 0.059 ( = 19)

P-Q conc		T	
before	after	before	after
14.0 ± 0.6 1.9 10	15.1 ± 0.5 1.7 ( = 10)	0.90 ± 0.02 0.07 ( = 10)	0.89 ± 0.02 0.07 ( = 10)
13.8 ± 0.4 1.2 10	13.6 ± 0.6 2.0 ( = 10)	0.95 ± 0.02 0.05 ( = 10)	0.94 ± 0.01 0.04 ( = 10)
12.2 ± 0.3 0.8 6	11.7 ± 0.2 0.5 ( = 6)	0.97 ± 0.03 0.07 ( = 6)	0.97 ± 0.03 0.06 ( = 6)
14.2 ± 0.3 1.0 10	13.9 ± 0.5 1.6 ( = 10)	0.91 ± 0.01 0.04 ( = 10)	0.92 ± 0.02 0.05 (n = 10)
14.8 ± 0.6 1.8 10	13.1 ± 0.3 1.6 ( = 10)	0.93 ± 0.02 0.05 ( = 10)	0.96 ± 0.02 0.06 ( = 10)

Table 12 Changes in rhythm and conduction following 92 digitalizations

	at rest	standing	exercise	After exercise		Total
				15 min. after	3 min. after	
sinus arrhythmia	30	7	0	18	9	64
sino-aurial block	0	0	0	1	0	1
auricular premature beats	2	0	0	1	0	3
ventricular " "	1	1	0	7	0	4
A V block I and II	2	1	0	3	3	9
Total	35	9	0	25	12	81

ization. In this subject the conduction time varied as 17—24—26 csec. before digitalization her P-Q interval was 18 csec. Her heart rate on the other hand was fairly similar before and after digitalization (127 beats/min before and 123 after). No other subject had a P-Q interval of such magnitude that one can speak of a block.

With lanatoside C in smaller doses (0.8—0.4 mg) there was no significant change in P-Q interval.

Three minutes after exercise the P-Q intervals both for men and women were still rather longer after the largest dose of lanatoside C. Before digitalization the men had a P-Q interval of 15.6 csec, and after 17.0 csec, the difference being probably significant ( $p = 0.02-0.01$ ). For women the corresponding figures were 15.0 and 16.2 csec ( $p = 0.02-0.01$ ). One digitalized subject (no. 52) three minutes after exercise had a P-Q interval of 30 csec before digitalization her figure was 18 csec. Her heart rate before and after digitalization was, respectively 90 and 94 beats/min. Subject no. 21 who had a P-Q interval of 26 csec immediately after exercise had the same interval three minutes after exercise.

Nor with the lower doses of lanatoside

C was there any significant change of the P-Q interval three minutes after exercise.

### B. Digtoxin

Immediately after exercise the P-Q interval in this group was 14.6 csec before digitalization, 16.1 csec after (table 10). The difference is not significant ( $p = 0.2-0.1$ ). Two subjects after digitalization had a considerably prolonged P-Q interval. One subject (no. 44) had before digitalization a P-Q interval of 17 csec and heart rate of 113 beats/min. After digitalization the P-Q interval immediately after exercise was 24 csec and the heart rate 120.

The second subject (no. 47) immediately after exercise had a P-Q interval of 19 csec before digtoxin and after digtoxin 42 csec, A-V block II with Wenckebach periods, which she also had at rest after digitalization.

Three minutes after exercise the P-Q interval was 15.1 csec before digtoxin and 17.1 after (S.D.  $\pm 6.12$ ) the difference, despite the large increase, being non-significant ( $p = 0.2-0.1$ ).

One subject (no. 44) who immediately after exercise had a P-Q interval of 24

caec, now had a P-Q interval of 17 caec at heart rate 116 against 16 caec at heart rate 95 before digitalization.

### C. Digoxin

From table 11 it will be seen that the P-Q interval before digoxin was 14.2 caec immediately after exercise and 14.8 caec three minutes after exercise. The corresponding figures after digoxin were 13.9 and 15.1 caec. The differences were not significant ( $p = 0.5-0.4$  and  $0.6-0.5$  respectively)

### Q-T interval

#### A. Lanatoside C

The Q-T interval indicated as an "index" will be seen in tables 9-10 and 11. At rest the predigitalization figures were 0.93 for men and 0.98 for women. After 1.6 mg of lanatoside C they were 0.90 and 0.92. The difference is significant for women ( $p = 0.01-0.001$ ) and not significant for the male subjects ( $p = 0.2-0.1$ ).

In standing position and during and after exercise the index did not differ significantly ( $p = 0.1-0.05$ ) either in women or men (table 9).

After smaller doses of lanatoside C (0.4-0.8 mg) the index at rest was  $1.00 \pm 0.06$  before and  $0.99 \pm 0.04$  after 0.4 mg, and  $1.0 \pm 0.09$  after 0.8 mg. The differences are not significant ( $p > 0.1$ ). In standing position the index was  $1.0 \pm 0.05$  before and, respectively,  $1.0 \pm 0.05$  and  $1.0 \pm 0.03$  after 0.4 and 0.8 mg lanatoside C ( $n = 9$ ) i. e. no difference.

During exercise the index was  $0.9 \pm 0.07$  before and, respectively,  $0.97 \pm 0.05$  and  $0.98 \pm 0.03$  after 0.4 and 0.8 mg. Here again there was no significant difference. Immediately after exercise the index was  $0.97 \pm 0.04$  before and, respectively,  $0.95 \pm$

$0.05$  and  $0.94 \pm 0.05$  after 0.4 and 0.8 mg ( $n = 9$   $p > 0.1$ ). Three minutes after exercise the indices were identical before, after 0.4 and after 0.8 mg lanatoside C, namely  $1.0 \pm 0.04$ ,  $1.0 \pm 0.05$  and  $1.0 \pm 0.04$ .

### Digitoxin

At rest the index was 0.94 before and 0.86 after digitoxin. The difference was highly significant ( $p < 0.001$ ). In standing position the figures were 0.95 before and 0.91 after digitoxin. This difference was not significant ( $p = 0.1-0.05$ ).

The index did not differ either during exercise. Immediately after exercise the figures were 0.93 before and 0.91 after digitoxin ( $p = 0.2-0.1$ ) and three minutes after exercise 0.99 and 0.94 the difference is significant ( $p = 0.01-0.001$ ) (table 10).

### Digoxin

As will be seen from table 11 the indices are practically identical before and after oral digoxin at rest, standing, and during and after exercise.

### The effect of cardioactive glycosides on the ST-T region

#### 1. ST-T reaction before digitalization

As appears from table I, the majority of cases exhibit an isoelectric or slightly elevated ST region and positive T wave  $> 0.1$  mV at rest, standing, and in conjunction with exercise. A small number were grouped as 1-2 in standing, during and after exercise. (For explanation of symbols see fig 7). Four other cases had T wave changes of type 4-5 in standing position. In conformity with earlier opinions (Michel 1934, Bengtsson 1956 and Sandberg 1961) this has been regarded as a non-pathological reaction.



# I ECG before digitalization (n=62)

Type of change	Resting	Standing	During exercise	After exercise	
				Immed.	3 min.
0	61	50	55	58	55
1—2	1	8	7	4	7
4		2			
5		2			

## 2 ST T changes induced by digitalis

### A. Lanatoid C 1.5 mg

The ST T changes arising after injection of 1.5 mg lanatoside C are shown in tables 13 14 15 16 and 17

#### At rest

The height of the T waves in the various leads before and after administration of lanatoside C will be seen from table 13. The T waves were throughout lower after digitalization, the difference before and after digitalization being highly significant ( $p < 0.001$ ).

The ST T changes and their distribution in different leads after the digitalization are shown in table 14. Most electrocardiograms were unchanged and when changes were recorded they were generally mild.

This table shows the number of subjects who revealed changes in any lead after digitalization. They are divided into groups as follows and as shown in fig. 7. Group 0 no changes, groups 1—2 changes of types 1—2, groups 3—10 changes of types 3—10 and group 11. It will be seen that 64% of the subjects had unchanged ST T regions, 14% mild changes of types 1—2, 20% changes of types 3—10 and only 2% changes of type 11 (table II).

#### Standing

After 3 minutes standing, changes due to digitalization were recorded both in a greater number of subjects and of more types as at rest. The results are shown in table 15.

## II ST T changes at rest

Group	No. of subjects	%
0	27	64
1—	6	14
3—10	8	20
11	1	2
Total	42	100

## III Standing

Group	No. of subjects	%
0	5	12
1—2	13	31
3—10	22	52
11	2	5
Total	42	100

In only 12% of the subjects was the ST T region unchanged, 31% had changes of types 1—2, 52 % changes of types 3—10. Here again there were few (5 %) who exhibited an ST region of type 11. The changes were most pronounced in leads II and CH<sub>1</sub> (table III)

#### *During exercise*

Table 16 shows that lanatoside C in this dose caused more pronounced ST T changes than at rest and standing.

In only one subject was the ST T region unchanged. Changes of types 1—2 were recorded in 17 % of the subjects, types 3—10 in 74 % type 11 only in 7 %. The ST T changes were most pronounced in lead CH<sub>1</sub>.

#### *IV During exercise*

Group	No. of subjects	%
0	1	2
1—2	7	17
3—10	31	74
11	3	7
Total	42	100

#### *V Immediately after exercise*

Group	No. of subjects	%
0	25	60
1—	11	26
3—10	5	12
11	1	2
Total	42	100

#### *After exercise*

Immediately after exercise 60 % had unchanged ST T region, in 26 % digitalization caused changes of types 1—2, in 12 % more pronounced changes.

Three minutes after exercise the changes had become more accentuated, 29 % having changes of types 3—10 19 % of types 1—2, and 45 % no changes in the ST T region.

Table 13 shows the height of the T wave at rest and during the post-exercise period. At rest the T waves were throughout lower after digitalization, and likewise after exercise, the differences being highly significant ( $p < 0.001$ )

Prior to digitalization the T waves were higher immediately after exercise than at rest, but returned to the resting value 3 minutes after exercise. The same pattern was observed after digitalization, the T waves immediately after exercise being higher than at rest but 3 minutes after exercise again of the same height as at rest.

The same tendency is seen from tables V and VI. Immediately after exercise 26% exhibited changes in group 1—2 and 12% in group 3—10, whereas 3 min. after exercise 19% had changes in group 1—2 and 29% in group 3—10.

#### *VI 3 min after exercise*

Group	No. of subjects	%
0	19	45
1—2	8	19
3—10	12	29
11	3	7
Total	42	100

**Table 13** Height of T wave in mm at rest and in the postexercise period (1 mm = 1 mV) before and after 1.6 mg Lanatoside C ( $M \pm S.E.$ , S.D.)

Lead	Rest		Immediately after exercise		3 min after exercise	
	before	after	before	after	before	after
I	$2.9 \pm 0.2$ 0.9 (n = 41)	$1.8 \pm 0.14$ 0.9	$3.2 \pm 0.21$ 1.2 (n = 36)	$2.1 \pm 0.17$ 1.0	$2.5 \pm 0.13$ 0.9 (n = 39)	$1.5 \pm 0.16$ 0.9
II	$3.5 \pm 0.21$ 1.8 (n = 41)	$1.8 \pm 0.24$ 1.5	$4.5 \pm 0.36$ 2.1 (n = 32)	$3.1 \pm 0.28$ 1.6	$3.5 \pm 0.24$ 1.5 (n = 34)	$2.2 \pm 0.31$ 1.8
CR2	$8.9 \pm 0.61$ 3.8 (n = 38)	$6.5 \pm 0.61$ 3.7	$11.6 \pm 0.67$ 3.6 (n = 32)	$8.4 \pm 0.60$ 3.4	$9.0 \pm 0.51$ 3.0 (n = 33)	$6.4 \pm 0.54$ 3.1
CR5	$7.1 \pm 0.42$ 2.7 (n = 38)	$4.3 \pm 0.49$ 3.1	$9.14 \pm 0.44$ 2.6 (n = 31)	$6.5 \pm 0.49$ 2.7	$6.9 \pm 0.40$ 2.4 (n = 30)	$3.9 \pm 0.42$ 2.2
CR7	$5.1 \pm 0.25$ 1.6 (n = 41)	$2.9 \pm 0.28$ 1.8	$5.6 \pm 0.29$ 1.7 (n = 32)	$3.3 \pm 0.35$ 1.9	$4.3 \pm 0.35$ 1.8 (n = 35)	$2.4 \pm 0.28$ 1.7

#### B. Lanatoside C 0.4 and 0.8 mg

Lanatoside C in doses of 0.4 mg intravenously changed the ST T region to a very much smaller extent than in doses of 1.6 mg.

At rest two subjects showed changes of types 1—2 (n = 8) in standing position one subject had changes of type 3 and one of type 4.

During exercise this small dose of lanatoside C caused ST T changes in four subjects (n = 8) in three of whom types 1—2 and in the fourth type 3. Immediately after exercise no ST T changes were recorded while 3 minutes after exercise one subject showed changes of type 2.

The changes in the ST T region caused by 0.8 mg of lanatoside C intravenously are set out in tables 18, 19, 20 and 21. The

tendency is the same as after 1.6 mg, but the changes are perhaps slightly less pronounced. But all subjects in this group (n = 9) exhibited ST T changes during exercise which were not recorded before digitalization. The changes were most pronounced in lead CH<sub>1</sub>. After exercise the ST T changes quickly receded.

#### C. Digitaloxin

The types of ST T changes caused by digitaloxin per os are shown in tables 22, 23, 24 and 25.

##### At rest

At rest (table 22) the conditions were roughly similar to those after digitalization with 1.6 mg of lanatoside C. 61% of

the subjects showed no changes, 9 % had changes of types 1—2, and 13 of types 3—10. Changes of type 11 were observed in four subjects (17 %)

#### VII. At rest

Group	No. of subjects	%
0	14	61
1—2	2	9
3—10	3	13
11	4	17
Total	23	100

#### Standing

After digitoxin the ST T region changed in largely the same way as after lanatoside C. It will be seen from table 23 that the changes were more common and of a more pronounced kind than at rest.

No changes were shown by 30 % changes of types 1—2 by 18 % types 3—10 by 30 % type 11 by 22 %

#### VIII. Standing

Group	No. of subjects	%
0	7	30
1—2	4	18
3—10	7	30
11	5	22
Total	23	100

#### During exercise

The results are presented in table 24

They do not differ appreciably from those for lanatoside C.

Digitoxin provoked changes within the ST T region in 91 % of the subjects, of types 3—10 in 69 %

#### IX. During exercise

Group	No. of subjects	%
0	2	9
1—2	5	22
3—10	16	69
11	0	
Total	23	100

#### After exercise

During the post-exercise period (table 25) the changes induced by digitoxin quickly receded. Neither immediately nor 3 minutes after exercise was there any appreciable difference from the observations during the post-exercise period after lanatoside C.

The percentual distribution of ST T types shown in tables X and XI.

#### X. Immediately after exercise

Group	No. of subjects	%
0	10	44
1—2	9	39
3—10	4	17
11	0	
Total	23	100

# XI 3 minutes after exercise

Group	No. of subjects	%
0	9	39
1—2	8	35
3—10	6	26
11	0	
Total	23	100

## D Digoxin

The digoxin group comprises comparatively few subjects ( $n = 10$ ). The results are shown in tables 26, 27, 28 and 29. The tendency is the same as after lanatoside C and digitoxin, the ST-T changes being comparatively few at rest and more nume-

rous in standing. During exercise all digoxin subjects exhibited ST-T changes.

## Summary

Ninety-two tests were made with different digitalis gluconides. Digitalis gluconides caused ST-T changes at rest in 30 cases (32 %) standing in 69 cases (75 %) during exercise in 82 cases (89 %). Immediately after exercise the number of cases with ST-T changes was 34 (37 %) and 3 minutes after exercise 44 (48 %). The types of changes are tabulated below (table XII).

## Localization

It is quite clearly apparent from tables 14—29 that ST-T changes most often occur in the left precordial leads and least often in the right precordial leads. The percentual distribution is shown in table XIII.

# XII

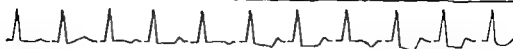
Type	Rest %	Standing %	During exercise %	Immediately after exercise %	3 minutes after exercise %
0	68	25	11	63	52
1-2	13	25	27	25	21
3-10	14	42	58	10	23
11	5	8	4	2	4
Total	100	100	100	100	100

# XIII

Load	Rest		Standing		Exercise		Post exercise			
							0 min		3 min	
	%	%	%	%	%	%	%	%	%	%
I	15	17	38	16	—	—	13	14	13	9
II	21	24	36	25	—	—	29	30	28	20
CRACH	3	3	17	7	23	11	2	2	5	4
CRACH <sub>4</sub>	14	16	33	14	33	24	12	20	24	17
CRACH <sub>4</sub>	17	19	38	16	65	29	19	21	34	24
CRACH	15	21	52	22	81	36	20	15	37	26
Total	89	100	236	100	222	100	95	100	141	100

*Types and distribution of ST and T changes after administration  
of lanatide C (1.6mg)  
(n = 42)*

14 At rest



	0	1	2	3	4	5	6	7	8	9	10	11
33		9										
28		3	2	7	1		1					
41					1							
37			1	1	2	1						
35		1	2	2	1							1
32		4	3	1				1				1

15 Standing

19	20		2	8	3	2	4	1	1			1
9	10	2		8	3							2
34	2			5	1							
26	3			8	1		1	1	1			1
22		2		4	3		2	1	1			1
14	9	3		3	5	1			1			4

16 During exercise

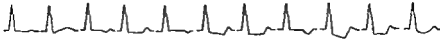
25	2	4	1	5			2					
14	4	3		7		1	10		2			
5		7		8		3	12		2	1		1
1		8		14		3	8		2	1		3

17 After exercise

0'	3'	0	3	0'	3'	0	3'	0'	3'	0'	3	0'	3	0'	3	0'	3'
35	35	3	2	2	2		3		3								
30	27		1	7	4	1	2	3	7		1					1	
40	40		1		1												
35	27	1		5	6	1	1		6				1				5
33	24	1	1	7	4				8		3					1	2
35	22		1	5	5		1	3	7		3					1	3

*Types and distribution of ST and T changes after administration  
of lanatoside C (0.8 mg)  
(n = 9)*

*Table 18 At rest*

													change total
lead	0	1	2	3	4	5	6	7	8	9	10	11	
I	8	1											1
II	8			1									1
CR2	8				1								1
CR4	8				1								1
CR5	8		1										1
CR7	8		1										1

*Table 19 Standing*

I	5	3	1										4
II	2	1	1	4	1								7
CR2	6			2					1				3
CR4	5			2	1		1						4
CR5	4	1		2	1		1						5
CR7	1	3	1	4									8

*Table 20 During exercise*

CH2	5	2		1	1								4
CH4	4	1	1	1	1				1				5
CH5	4	1	2	1					1				3
CH7	0	6	1	1					1				9

*Table 21 After exercise*

	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'
I	5	7		2														
II	8	7			1	1		1										2
CR2	9	7				1		1									1	2
CR4	9	7					2											2
CR5	9	7					2											2
CR7	9	6		2		1												3



*Types and distribution of ST and T changes after administration of digitoxin per os  
(n = 29)*

22 At rest



	0	1	2	3	4	5	6	7	8	9	10	11
19		3										1
19			2									
22					1							
16			4	1								2
16		1	4									2
15			3	1	1							3

23 Standing

	0	1	2	3	4	5	6	7	8	9	10	11
17		4		1								1
8		4	1	1	2	1	1					3
20		1	1					1				
15		1	3	1					1		1	
12		1	4	1	1		1		1			1
10		4	2	1		1			1			3

24 During exercise

	0	1	2	3	4	5	6	7	8	9	10	11
18		1		1			1					
7			1	2	4		2			3	2	
6			2		3		3	1			2	
2			1		3		3	6		1	2	
			4									

25 After exercise

0'	3'	0'	3'	0'	3'	0'	3'	0'	3'	0'	3'	0'	3'	0'	3'	0'	3'
17	19	3	1	1		1	2	1		1							
11	14			9	4	1		4		1							
21	22			1	1									1		1	
19	16			3	3		1	1						1	1		1
15	11			4	7	1		1		1	2						1
13	11			8	5					3							

*Types and distribution of ST and T changes after administration of digoxin per os  
(n = 10)*

Table 26 At rest


													changes total
lead	0	1	2	3	4	5	6	7	8	9	10	11	
I	9		1										1
II	8	1	1										2
CR2	9	1											1
CR4	9				1								1
CR5	8				1								2
CR7	9				1								1

Table 27 Standing

I	7	2			1								3
II	6	3			1								4
CR2	9	1											1
CR4	6	2		1	1								4
CR5	7	1		1			1						2
CR7	8	1					1						3

Table 28 During exercise

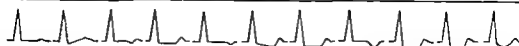
CHL	10												0
CH4	7		3										3
CH5	3		4		2						1		7
CH7	3	3			3						1		

Table 29 After exercise

lead	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'	0'	5'
I	9	9			1				1								1	1
II	8	8			1				1								1	1
CR2	10	10											1	1	2	2		
CR4		9			2												3	1
CR5	7	8			1						1				2	1	3	2
CR7	8	7			1	1					1				1	1	2	3

*Types and distribution of ST and T changes after administration of digtoxin per os  
(n = 23)*

22 At rest



d	0	1		3	4	5	6	7	8	9	10	11
	19	3										1
	19		2		2							
2	22				1							
4	16		4	1								2
5	16	1	4									2
7	15		3	1	1							3

23 Standing

	17	4		1								1
	8	4	1	1	2	1	1					3
2	20	1	1					1				
4	15	1	3	1					1		1	
5	12	1	4	1	1		1		1			1
7	10	4	2	1		1			1			3

24 During exercise

2	18	1	1	1			1					
4	7		2		4		2	1		3	2	
5	6		1		3		3	6			2	
7	2	2	4		3		3	6		1		

5 After exercise

		0' 3	0' 3	0' 3	0' 3	0' 3	0 3	0 3	0' 3	0' 3	0' 3	0' 3	0' 3	0' 3
		17 19	3 1	1		1	2 1			1				
		11 14		9 4	1	2 4			1					
2		21 22		1 1									1	1
4		19 16		3 3		1 1						1	1	1
5		13 11		4 7	1	1			1	1				1
7		13 11		8 3		2			3 2					

Fig. 2.

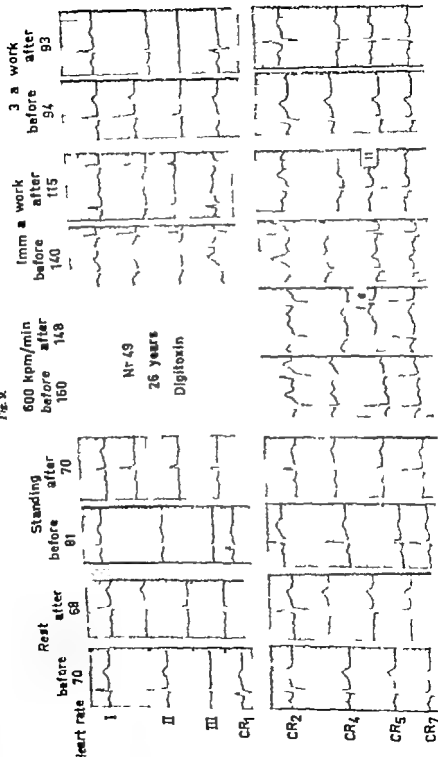


Fig. 8. The figures on the ECG tracings in figs. 8, 9, 10 and 11 correspond to the types of ST and T changes shown in fig. 7

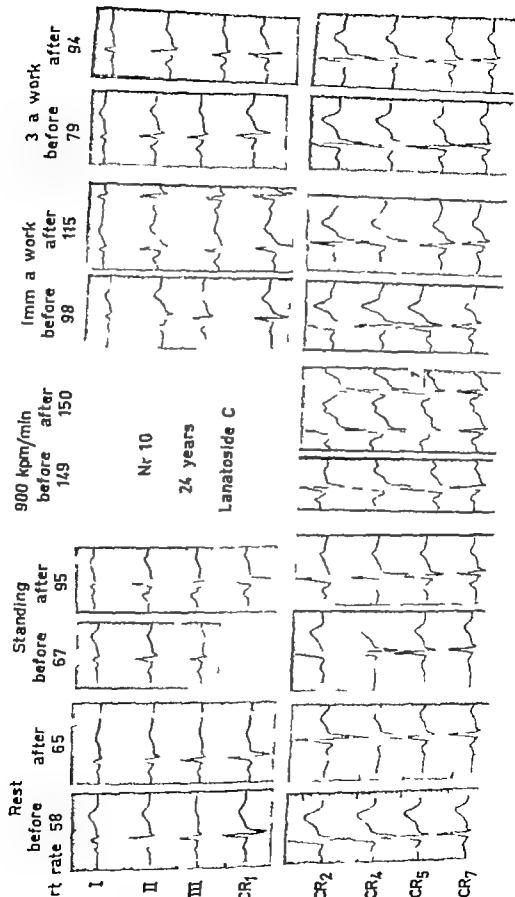


Fig. 11

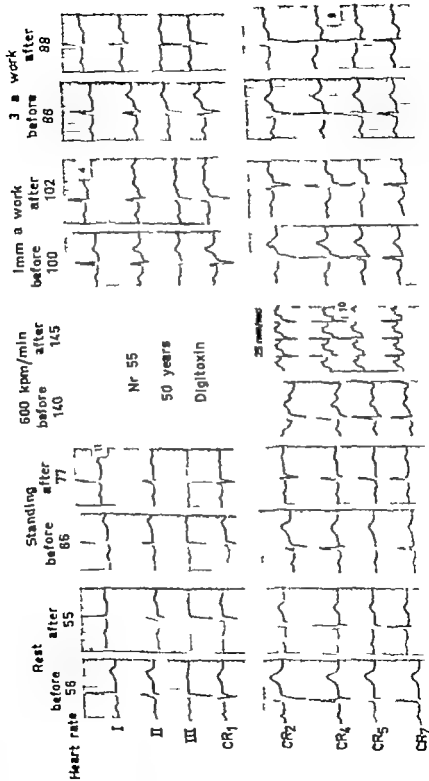
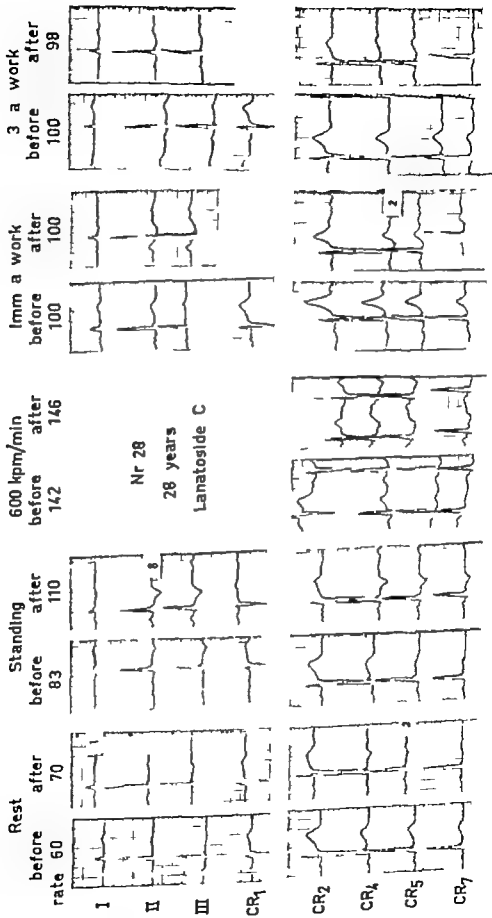


Fig. 10



is even in excessive doses (e. g. in attempted suicide) but A-V block II with Wenckebach periods may occur (Hedley 1943 Bickel et al., 1951 Vuletic & Ivancic, 1952, MacMillan & Bellet, 1952)

Bengtsson (1957) found that during heavy exercise at a heart rate of around 170 per minute the P-Q interval dropped to the same level, viz. 0.11 second in all age groups (in his material up to 40 years) irrespective of the initial value at rest, or of age and sex.

After digitalis the P-Q interval was prolonged at rest and after exercise in my material there were isolated cases of A-V block I and one of A-V block II. At high loads (600 kpm/min for women, 900 kpm/min for men) the P-Q intervals were exactly the same as before digitalization, even in the subject with A-V block II. Thus in healthy subjects digitalis had a moderate effect on the P-Q interval at rest and after exercise, but the P-Q interval during exercise was unaffected by digitalis. Nor was there other than low grade block in my material.

Total block has admittedly been produced in dogs after very large doses of digitalis (Moe & Mendez, 1952, Dearing & al., 1943) but clearly the doses used by healthy persons have been too small (although toxic) to produce block, which is liable to appear as a sign of intoxication in the diseased heart

#### THE Q-T interval

Handbooks report a shortening of the Q-T interval as a constant finding in cases of digitalization (e. g. Lepeschkin, 1951 Goldberger 1953 Gardberg, 1957) Cheer & Druvaud (1931) showed that digitalis shortened the Q-T interval and they considered this actually to be the most sensitive indicator of digitalization. Larsen & Nielsen (1936, 1937) Wedd &

Blair (1947) Sandberg et al. (1950) MacMillan & Bellet (1952) and Rothlin (1954) also found that digitalis definitely shortened the Q-T interval. Rothlin says that this is the first objective sign of the effect of digitalis, and MacMillan & Bellet that it may be a good way of differentiating ST changes caused by coronary insufficiency from those caused by digitalis.

Doubt on this score has been expressed from several quarters. Geiger et al (1941) found shortening of the Q-T interval in some cases, but of an extremely temporary nature. Ruskin & Décherd (1948) found in animal experiments that small doses prolonged the Q-T interval while large doses shortened it. Koelbing (1950) in human experiments, found that small doses had no effect on the Q-T interval, while large doses produced fairly transient changes. Dönhardt (1951) reported a shortening of the Q-T interval in roughly half of his subjects.

Yu et al. (1952) found no direct shortening of the Q-T interval, but a lowering of the QT/TQ ratio at rest though none during exercise. These are the only authors to have recorded the ECG during exercise. Woodbury & Hecht (1952) found that cardioactive glucosides had no effect on the depolarization of the frog heart but shortened the repolarization time, which would explain the shortening of the Q-T interval.

As was stated in the section on measuring methods, measurement of the Q-T interval is difficult. After for example, tilting and exercise the positive after potential increases (Sjöstrand, 1951) This happens also after digitalis, as I found in my experiments. This positive after potential, which is added to the T wave, makes it extremely difficult to decide when the T wave ends, especially at high frequencies. I am aware that the use of an index, i. e. introducing still another measurement,



## Discussion

Irregular or ectopic impulse formation and change of impulse conduction have been observed in patients in conjunction with more or less pronounced digitalis intoxication: sinus arrhythmia, wandering pacemaker, sino-auricular standstill, nodal rhythm, atrio-ventricular dissociation, A-V block of all degrees, premature beats see *inter alia* Burwell & Hendrix, 1950; Pick, 1957.

The effect on the heart rate has been dealt with earlier and will therefore be disregarded here.

*Sinus arrhythmia* following upon digitalization has been regarded as may be a sign of light digitalis intoxication (Bellet, 1963). Larsen & Nielsen (1936, 1937) found some cases of sinus arrhythmia after digitalization of healthy subjects.

In my series sinus arrhythmia induced by digitalis was recorded at rest in one third of the subjects, but in none during exercise. During the post-exercise period only 3 had sinus arrhythmia.

Among other rhythmic disorders in the sino-auricular node sino-atrial block was observed in one case immediately after exercise. Liebow & Feil (1941) found two cases of sino-atrial block in healthy digitalized persons immediately after exercise. In cardiac patients sinoatrial block has been recorded in conjunction with digitalis intoxication (Flaxman, 1948; Herriman et al. 1944; Burwell & Hendrix, 1950).

In my material occasional supraventricular premature beats were recorded after digitalis, and only a few ventricular premature beats, of which some during exercise at the lightest load. No premature beats were recorded at higher loads. Case 15 who had ventricular premature beats even before digitalization, was not included in table 12. This person had ventricular premature beats at rest, standing

and during exercise except at the heaviest load. Ventricular premature beats returned some time after exercise. Digitalization did not increase the frequency of premature beats. This person was and is completely healthy but was a heavy smoker. He was re-examined six months later when he had stopped smoking, and then exhibited no ventricular premature beats either before or after digitalization.

In persons with healthy hearts premature beats, particularly ventricular, are extremely rare after digitalization, even in toxic doses (MacMillan & Bellet, 1952; Sagall & Wolff, 1949; Larsen & Nielsen, 1936; Hedley, 1943). In persons who have taken enormous doses of digitalis for suicidal purposes, only occasional or no premature beats have been recorded (Bachman, 1960; Bickel et al., 1951; Vuletic and Ivancic, 1952; Nordström-Öhrberg, 1962). Yu et al. (1952) found two cases of ventricular premature beat after exercise in healthy subjects in conjunction with digitalis during exercise; no premature beats were recorded. Moe & Mendez (1951) showed, however, that ventricular arrhythmia could be provoked in dogs with doses 70–80 % of the lethal dose (therapeutic doses are around 35 % of the lethal dose).

The A-V conduction is variously affected by digitalis, from no effect at all (Apter et al., 1944; Koelbing, 1950; Geiger et al., 1941; Yu et al., 1952) to prolongation of P-Q interval (Larsen & Nielsen, 1936; Sagall & Wolff, 1949; Ruskin & Déchard, 1948) to block of mild degree, i.e. A-V block I and A-V block II with Wenckebach periods (Gilchrist, 1958). These low grade blocks diminish after exercise, while high grade blocks (A-V block II with constant conduction time and A-V block III) increase after exercise (Gilchrist, 1958; Landegren, 1964). In healthy persons total block never occurs after digitali-

23% displaying no change. The percentual distribution of the changes was usually similar for the different glucosides. The standing heart rate was higher than the supine. In the lanatoside C cases, more over the heart rate rose still further after digitalization. The standing ST T reaction was, however fairly similar for all three glucosides despite the difference in standing heart rate between the lanatoside C cases on the one hand and the digitoxin and digoxin on the other. The rise of heart rate from supine to standing position may have been sufficient to accentuate the existing ST T changes and provoke new ones. But it would be expected that the lanatoside C subjects, who had a higher standing heart rate than the other subjects, would display more changes.

During exercise the ST T depression increased still further 89% of the subjects showing changes after digitalization, and 58% of types 9-10. Immediately after exercise the height of the T waves increased and the ST T changes decreased. Three minutes after exercise the ST T changes increased again.

Sjöstrand (1950 a and b) reported that the amplitude of the T was normally decreases and the ST depression increases with rising heart rate and that the decrease in T-wave during work is just the reverse of the effect observed in the horizontal position immediately after the cessation of work. Between one and two minutes after the cessation of work, however the amplitude of T-wave again decreases.

It is reasonable to suppose, therefore that a major reason for the heavy accentuation of the post-digitalization ST T changes during exercise is the rise of heart rate. This sympathicotonic element in the electrocardiographic picture is evident also in the circumstance that both ST and T regions in this material were normalized

immediately after exercise. This is in agreement with the observations in cases of high sympathetic tone (Holmgren et al. 1957 1959). To regard the electrocardiographic changes induced by digitalis as solely due to increased sympathetic tone, on the other hand, is hardly realistic. Koelbing (1950) for example, proved that digitalis-induced changes are not affected by ergotamine as are the T-wave changes of sympathicotonic origin (Nordenfält 1941 Björck 1947).

Laebow & Feil (1941) found that 4 digitalized subjects of 14 in the post-exercise period displayed ST T changes which were considered pathological though without specification of their degree and Yu et al. (1952) that 7 of 20 subjects during exercise and in the post-exercise period had pathological ST T changes after digoxin, but again without characterization of type.

The changes on the ECG particularly in the ST T region, produced by cardioactive glucosides have occasionally been considered to be proportional to the degree of digitalization. On this assumption attempts have been made to use the ECG for human bioassay (Pardee 1923, Gold et al. 1941 Tandowny et al 1944) found the electrocardiographic criteria of the effect of digitalis fully reliable, and Wood (1956) writes "The electrocardiogram offers by far the most reliable evidence of digitalis saturation".

As early as 1941 Geiger et al. concluded "that the electrocardiogram has no practical clinical value for the quantitative estimation of the digitalis saturation of patients" and this is the current view (de Graff 1946, Koelbing 1950 Dönhardt 1951 Simon 1952, and others).

From the present experiments it is evident that the ST T changes after 0.4 mg lanatoside C were extremely small, and that after 0.8 mg lanatoside C intrave-

may affect the result still more. But the use of an index is simple and practical, and there is no particular advantage in calculating the corrected Q-T interval in accordance with one of the customary formulas.

In my experiments the index" was lower at rest i.e. the Q-T interval was shorter after digitalis medication as also observed earlier at rest. On the other hand digitalization did not shorten the Q-T interval in the standing position nor during or after exercise. Why this should be so is difficult to explain.

But shortening of the Q-T interval is of little value in diagnosing the effect of digitalis in clinical practice, for one usually has no knowledge of the Q-T interval before digitalization. "Normal" Q-T intervals vary within wide limits. Neither in the literature referred to in this paper nor in my experiments was the shortening of the Q-T interval (when it occurred at all) of such magnitude as to be a conspicuous feature.

### *The ST and T region*

Cohn & Fraser reported in 1913 T wave changes in the electrocardiogram as a result of digitalization. Since then the literature has abounded with accounts of the effect of digitalis on the ST T region, both in heart patients (Pardee 1923 Strauss & Katz 1934 Luten 1936 Stewart & Watson 1938 Eichna et al. 1943 Tandowsky et al. 1944 Rothlin 1954) and in healthy persons (Routier & Puddu 1935 Larsen & Nielsen 1936 1937 Geiger et al. 1941 Koelbing 1950 Donhardt 1951 Beers et al. 1951 MacMillan & Bellet 1952 and Tiliakos 1953) Routier & Puddu (1935) found that changes in the ST T region occur as often in healthy as in cardiac patients.

All these studies have related to the ECG at rest, and almost all authors state that changes within the ST T region caused by digitalis cannot be distinguished from those seen in coronary insufficiency.

Attempts have been made to arrive at a qualitative basis for diagnosing ST T changes as due to digitalization and also to grade the changes (Lepeschkin 1951 MacMillan & Bellet 1952, Goldberger 1953) MacMillan & Bellet described different types of changes which they classified in four grades from slight T wave depression to very pronounced ST T depressions. One type of change was a depressed, smoothly concavely rounded, "cup-shaped" ST segment. It is this form which is often regarded as typical of digitalization. Goldberger (1953) reported this form to be comparatively rare. In the present study it is classified as type II and appears in only a few per cent of all cases. The usual reference in the literature, however is to typical sagging of the ST segment.

Yet it is difficult to indicate any particular change as typical of digitalis glycosides. This is apparent from the findings in this study in which eleven types are described. In the author's opinion it is more reasonable to speak of nonspecific ST T changes for as Friedberg & Zager (1961) state, "although characteristic ST T wave changes have been described following digitalis, the changes are not always characteristic".

In the present study the ST T changes at rest were comparatively slight, 68 % of subjects displaying no change 13 % having changes of types 1-2, 14 % more pronounced changes, and only 5 % showing the concave cup-shaped type referred to above.

In standing position the changes within the ST T region were more common and more pronounced, 42 % of all subjects having changes of types 3-10 and only

In the present study  $G$  was determined at rest and standing before and after digitalis. The results are shown in table 30.

As is seen, AQRS was unchanged after digitalis, which accords with the fact that digitalis glycosides do not affect the depolarization. Likewise the direction of the vector AQRS shows no statistically significant changes.  $G$  on the other hand, declines, being 8.4 units after digitalization against 11.7 before, a highly significant difference ( $p < 0.001$ ). Before digitalis  $G$  was smaller standing than prone. After digitalis it decreased still further. Before digitalis  $G$  was 7.6 units in standing, after digitalis 6.0 units, a probably significant difference ( $p = 0.02-0.01$ ).

The direction of  $G$  showed only slight variations, the differences before and

after digitalization being non-significant ( $p > 0.1$ ).

The magnitude of  $G$  is influenced by many non-pathological factors. Apter et al. (1944) showed that digitalis reduced the gradient but did not change its direction. A high heart rate likewise reduces the gradient (Rowen 1957). The reduction of the gradient causes a shift of the ST segment. The combination of digitalis and high heart rate means that one must usually count on ST shifts due to diminution of the magnitude of the gradient under these conditions.

In ischaemic heart disease, on the other hand, the gradient is both diminished and its direction is changed, as shown in conjunction with positive hypoxaemia tests by Björck et al. (1948).

Table 30

	No. of subjects	AQRS units	AQRS degrees	$G$ units	$G$ , degrees
rest, before digitalis	27	$5.5 \pm 0.34$ 2.9	$+58 \pm 5.4$ 28	$11.7 \pm 0.62$ 3.5	$+45 \pm 4.6$ 24
rest, after digitalis	27	$5.5 \pm 0.54$ 2.8	$+61 \pm 4.2$ 22	$8.4 \pm 0.64$ 3.4	$+49 \pm 3.4$ 18
standing before digitalis	27	$4.5 \pm 0.44$ 2.3	$+61 \pm 10.6$ 35	$7.6 \pm 0.65$ 3.4	$+43 \pm 4.7$ 23
standing, after digitalis	27	$4.5 \pm 0.47$ 2.4	$+62 \pm 9.4$ 47	$6.0 \pm 0.37$ 1.8	$+39 \pm 5.9$ 30

ously and 1.25 mg digoxin orally the changes were rather more pronounced and more frequent. After 1.6 mg lanatoside C and after digitoxin the changes were more common. On the other hand changes occurred after very varying doses, and subjects who had subjective symptoms after digitalization did not exhibit more pronounced changes than the asymptomatic subjects. So far as can be judged from this material it is impossible to decide the degree of digitalization on the basis of the electrocardiogram.

There has been much controversy about the genesis of the ST-T changes. The apparent similarity between ST changes caused by digitalis and those seen in coronary disease has earlier been regarded as an indication that digitalis really causes a decline of the coronary blood flow (Büchner 1934 Levy et al 1940 Sandberg et al 1950). This was thought to lead to cardiomyocardial necrosis which in animal experiments has been observed after massive doses of digitalis (Büchner 1933 1934 Bauer 1934 Dearing et al 1943b). These doses were, however many times larger than therapeutic doses. After therapeutic doses these changes were not observed. Neither Dearing et al. nor La Due (1941) found any changes in the heart muscle of dogs which had been given a full digitalization dose daily for one month.

In fact it has been convincingly proved that the coronary blood flow is not diminished by digitalis (Bing et al. 1950 Bing & Daley 1951 Wegria 1951). Exercise tests of the kind described in this study do not indicate any impairment of the myocardial function.

Woodbury & Hecht (1952) using a microelectrode technique, studied the effect of digitalis glucosides on the frog heart *in situ*. They found that cardioactive glucosides did not affect the depolarization but did affect the repolarization,

the duration of the membrane action potential being markedly shortened. Surface electrocardiograms recorded from the ventricular surface exhibited ST changes which could be correlated to simultaneous single fibre records. The uniform shortening of the repolarization thus resulted in depression of ST and shortening of QT.

The effect of cardioactive glucosides on the various metabolic processes and on the sodium and potassium concentration, by which the action potential is ultimately affected, lie outside the scope of the present study.

The changes occurred principally in the left precordial leads, which conforms with previous reports. Beers et al. (1951) found the most pronounced changes from digitalis in the left precordial leads for horizontal hearts and in the right precordial leads for vertical hearts. MacMillan & Bellet (1952) state that the largest ST-T depressions in conjunction with digitalization occur in the leads where R is greatest, and Paul Wood (1956) sums up by saying that the ST-T changes are most pronounced in leads V 4-6.

### *Ventricular gradient*

The concept of the ventricular gradient was created by Wilson and later developed by Ashman (1943 Ashman & al. (1943 a and b) and Gardberg & Ashman (1943).

The ventricular gradient (G) represents in the frontal plane the projection of the net electrical effects of differences in rates of repolarization in various areas of the ventricular muscle.

There has been controversy about the use of G since G varies enormously from individual to individual and even within the same individual. But it has been stated that G is of value for the study of serial electrocardiograms (Gardberg 1957).

In the present study  $G$  was determined at rest and standing before and after digitalis. The results are shown in table 30.

As is seen, AQRS was unchanged after digitalis, which accords with the fact that digitalis glycosides do not affect the depolarization. Likewise the direction of the vector AQRS shows no statistically significant changes.  $G$  on the other hand, declines, being 8.4 units after digitalization against 11.7 before a highly significant difference ( $p < 0.001$ ). Before digitalis  $G$  was smaller standing than prone. After digitalis it decreased still further. Before digitalis  $G$  was 7.6 units in standing, after digitalis 6.0 units, a probably significant difference ( $p = 0.02-0.01$ ).

The direction of  $G$  showed only slight variations, the differences before and

after digitalization being non-significant ( $p > 0.1$ ).

The magnitude of  $G$  is influenced by many non-pathological factors. Apter et al. (1944) showed that digitalis reduced the gradient but did not change its direction. A high heart rate likewise reduces the gradient (Rowen 1957). The reduction of the gradient causes a shift of the ST segment. The combination of digitalis and high heart rate means that one must usually count on ST shifts due to diminution of the magnitude of the gradient under these conditions.

In ischemic heart disease, on the other hand, the gradient is both diminished and its direction is changed, as shown in conjunction with positive hypoxemia tests by Blöck et al. (1948).

Table 30

	No of subjects	AQRS, units	AQRS degrees	$G$ , units	$G$ , degrees
rest, before digitalis	2	$5.5 \pm 0.34$ 2.9	$+58 \pm 3.4$ 28	$11.7 \pm 0.62$ 3.3	$+43 \pm 4.6$ 24
rest, after digitalis	27	$5.5 \pm 0.54$ 2.8	$+61 \pm 4.2$ 22	$8.4 \pm 0.64$ 3.4	$+49 \pm 3.4$ 18
standing, before digitalis	27	$4.5 \pm 0.44$ 2.3	$+61 \pm 10.6$ 55	$7.6 \pm 0.63$ 3.4	$+43 \pm 4.7$ 23
standing, after digitalis	27	$4.5 \pm 0.47$ 2.4	$+62 \pm 9.4$ 47	$6.0 \pm 0.3$ 1.9	$+39 \pm 5.9$ 30

ously and 1.25 mg digoxin orally the changes were rather more pronounced and more frequent. After 1.6 mg lanatoside C and after digitoxin the changes were more common. On the other hand changes occurred after very varying doses and subjects who had subjective symptoms after digitalization did not exhibit more pronounced changes than the asymptomatic subjects. So far as can be judged from this material, it is impossible to decide the degree of digitalization on the basis of the electrocardiogram.

There has been much controversy about the genesis of the ST-T changes. The apparent similarity between ST changes caused by digitalis and those seen in coronary disease has earlier been regarded as an indication that digitalis really causes a decline of the coronary blood flow (Büchner 1934 Levy et al 1940 Sandberg et al 1950). This was thought to lead to cardiomyocardial necrosis which in animal experiments has been observed after massive doses of digitalis (Büchner 1939 1934 Bauer 1934 Dearing et al 1943b). These doses were, however, many times larger than therapeutic doses; after therapeutic doses these changes were not observed. Neither Dearing et al nor La Due (1941) found any changes in the heart muscle of dogs which had been given a full digitalization dose daily for one month.

In fact it has been convincingly proved that the coronary blood flow is not diminished by digitalis (Bing et al 1950 Bing & Daley 1951 Wegria 1951). Exercise tests of the kind described in this study do not indicate any impairment of the myocardial function.

Woodbury & Hecht (1952) using a microelectrode technique studied the effect of digitalis glucosides on the frog heart in situ. They found that cardioactive glucosides did not affect the depolarization but did affect the repolarization,

the duration of the membrane action potential being markedly shortened. Surface electrocardiograms recorded from the ventricular surface exhibited ST changes which could be correlated to simultaneous single fibre records. The uniform shortening of the repolarization thus resulted in depression of ST and shortening of QT.

The effect of cardioactive glucosides on the various metabolic processes and on the sodium and potassium concentration, by which the action potential is ultimately affected, lie outside the scope of the present study.

The changes occurred principally in the left precordial leads, which conforms with previous reports. Beers et al. (1951) found the most pronounced changes from digitalis in the left precordial leads for horizontal hearts and in the right precordial leads for vertical hearts. MacMillan & Bellet (1952) state that the largest ST-T depressions in conjunction with digitalization occur in the leads where R is greatest, and Paul Wood (1956) sums up by saying that the ST-T changes are most pronounced in leads V<sub>4</sub>—6.

### *Ventricular gradient*

The concept of the ventricular gradient was created by Wilson and later developed by Ashman (1943 Ashman & al. (1943 a and b) and Gardberg & Ashman (1943).

The ventricular gradient (G) represents in the frontal plane the projection of the net electrical effects of differences in rates of repolarization in various areas of the ventricular muscle.

There has been controversy about the use of G, since G varies enormously from individual to individual and even within the same individual. But it has been stated that G is of value for the study of serial electrocardiograms (Gardberg 1957).

Table 32. Duration of ST T changes after administration of digitoxin per os.

	<1 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	Number of subjects
at rest	2	3	1	1		2	0	9
standing	4	4	2	5		1		16
during exercise	0	2	4	6	2	5	2	21
1 mo. after exercise	0	2	3	2	2	3	1	13
3 mo. after exercise	2	2	1	3	2	3	1	14

### B. Digitoxin

The duration of ST T changes induced by digitoxin is shown in table 32.

Changes at rest were recorded in 9 subjects, and in 2 of them were still present five weeks after completion of digitoxin medication.

In standing position changes were recorded in 16 subjects, persisting for up to three weeks and in one case up to five weeks. In the 21 subjects exhibiting ST T changes during exercise, the changes were still present in some cases six weeks after medication. In a check-up of these subjects some eight weeks after medication their ECG had returned to the picture before digitalization.

After exercise the duration of ST T changes was roughly the same as during exercise. One subject still exhibited changes six weeks after the end of medication.

### C. Digoxin

Ten subjects received a single dose of digoxin per os. The duration of the changes in the ST T region is shown in table 33.

The changes at rest persisted for less than one day. In standing position they lasted up to three days, and during and after exercise up to four days.

Table 33. Duration of ST T changes after administration of 1.25 mg digoxin per os.

	<1 day	1 day	2 days	3 days	4 days	5 days	Number of subjects
at rest	2	0					2
standing	2	1	1	1		0	5
during exercise	0	0	2	4	1		7
1 mo. after exercise	1			1	1		3
3 mo. after exercise		1		1	1	0	3



### Duration of ECG changes

As noted earlier from table 12 30 subjects developed sinus arrhythmia after administration of digitalis, 15 of whom had had lanatode C, 12 digitoxin and 3 digoxin. The arrhythmia was of comparatively short duration, lasting less than one day in 9 of the lanatode C cases, one day in 3 of them and two days in 3

The sinus arrhythmia induced by digitoxin remained for less than one week in 8 subjects and for one week in 4. In the 3 digoxin cases the arrhythmia lasted less than one day one day and two days, respectively. The premature beats recorded after digitalization were few in number and of occasional character. One subject developed sino-atrial block after lanatode C, lasting two days. A fairly pronounced prolongation of the P-Q interval was recorded in 7 subjects after lanatode C, persisting for less than one day in 2, one

day in 1 two days in 1 and four days in 3

After digitoxin, A V block II with Wenckebach periods lasting two weeks, was recorded in one subject after which the P-Q interval was considerably prolonged the prolongation persisting for three weeks. In another subject prolongation of the P-Q interval was recorded which persisted for less than one week.

The most common and important changes occurred, as noted earlier within the ST T region.

#### A. Lanatode C

The duration of changes within the ST T region caused by 1.6 mg of lanatode C is shown in table 31

At rest the changes persisted in some subjects for up to one day standing up to three days, during and after exercise up to five days.

Table 31 Duration of ST T changes after administration of 1.6 mg lanatode C intravenously

	<1 day	1 day	2 days	3 days	4 days	5 days	6 days	Number of subjects
at rest	11	4	0					15
standing	13	16	7	1	0			37
during exercise	3	1	13	17	6	1	0	41
Imm. after exercise	5	2	4		2	2	0	17
3 min. after exercise	9	2	3	4	3	2	0	23

Table 32 Duration of ST T change after administration of digitoxin per os.

	<1 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	Number of subjects
at rest	2	3	1	1		2	0	9
standing	4	4	2	3		1		16
during exercise	0	2	4	6	2	3	2	21
1 week after exercise	0	2	3	2	2	3	1	13
3 weeks after exercise	2	2	1	3	2	3	1	14

## B. Digitoxin

The duration of ST T changes induced by digitoxin is shown in table 32.

Changes at rest were recorded in 9 subjects, and in 2 of them were still present five weeks after completion of digitoxin medication.

In standing position changes were recorded in 16 subjects, persisting for up to three weeks and in one case up to five weeks. In the 21 subjects exhibiting ST T changes during exercise, the changes were still present in some cases six weeks after medication. In a check-up of these subjects some eight weeks after medication their ECG had returned to the picture before digitalization.

After exercise the duration of ST T changes was roughly the same as during exercise. One subject still exhibited changes six weeks after the end of medication.

## C. Digoxin

Ten subjects received a single dose of digoxin per os. The duration of the changes in the ST T region is shown in table 33.

The changes at rest persisted for less than one day. In standing position they lasted up to three days, and during and after exercise up to four days.

Table 33 Duration of ST T changes after administration of 1.25 mg digoxin per os.

	<1 day	1 day	2 days	3 days	4 days	5 days	Number of subjects
rest	2	0				0	2
standing	2	1	1	1			5
during exercise	0	0	2	4	1		7
1 week after exercise	1			1	1		3
3 weeks after exercise		1		1	1	0	3

## Discussion

In speaking of the duration of the effect of the various cardioactive glucosides one must distinguish between the *clinical effect* i.e. how long a clinical effect persists in respect of ventricular rate during atrial fibrillation or of recurrence of incompensation and the *effect on the electrocardiogram*. The duration of the clinical effect of digitoxin has been reported as 14–21 days, of lanatoside C and digoxin 3–5 days (Bellet 1963). Other authors report somewhat different durations. Lown & Levine (1954) put the duration of the digitoxin effect at more than 3 weeks, digoxin 4–7 days, and lanatoside C 2–3 days, while Hedlund (1952) gives a figure of up to 8 days for the effect of lanatoside C.

The duration of the effect on the ECG has earlier been studied only on the resting ECG. Campbell (1942) stated that A V block caused by *folium digitalis* passed off quickly within 2–3 days, and Bachman (1960) found that A V block disappeared within a few days. In the present study A V block persisted after digitoxin medication in one subject for two weeks.

ST T changes after *folium digitalis* have been reported to persist for one month (Larsen & Nielsen 1936 and Geiger et al. 1941). ST T changes caused by 16 mg lanatoside C disappeared within 16 hours

(Tandowsky 1942) while the same author found that ST T changes caused by digitoxin disappeared in two days. ST T changes in conjunction with attempted suicide with digitoxin disappeared after 11 days (Bickel et al. 1961). After digoxin ST T changes at rest persisted for one to two days (Helander & Östman 1960).

The duration of ST T changes is extremely individual particularly as regards the slow-acting glucoside, digitoxin, in the present study. Their duration at rest varied from less than one week to five weeks during and after exercise the duration was in some cases even longer.

These periods agree fairly closely with the period reported for excretion of digitalis which is short for lanatoside C (Bine et al. 1952) and longer for digitoxin. Friedman et al. (1950) demonstrated by biological methods that digitoxin, when given in a single dose, was excreted with the urine during 12–24 days.

Okita et al. (1953) and Okita (1957) using radioactive digitoxin, found that 60–80 per cent was excreted through the kidneys, and that during a period of up to 40 days (range 23–50 days) the excretion occurred in the form of unchanged digitoxin while metabolites were still being excreted some 80 days after the single dose. This is entirely in accordance with electrocardiographic observations of the duration of the ST T changes.

# REFERENCES

- Aldon A-O Hemmetskyld S O and Nielsen A., Skandinav. Arch. f. Physiologie 78 8, 1938.
- Aldon, A.-O and Nielsen, A. Skandinav. Arch. f. Physiologie 78 1 1938.
- Apter L., Ashman R. and Hall E. J Pharmacol. & Exper Therap. 82 227 1944.
- Ashman, R. Am. Heart J 26 495 1943.
- Ashman R., Byer E. Bayley R. H. Am. Heart J. 35 16 1949 a.
- Ashman, R., Gerdberg, M. and Byer E. Am. Heart J 25 36, 1943 b.
- ✓ Ashman R. Gerdberg, M. and Byer E. Am. Heart J. 26 473 1943.
- Backman M. Zeitschr Kreislaufforsch. 49 962, 1960.
- Barker S. B. and Semonson, R. H. J Biol. Chem. 158 535 1941.
- Bauer H. Arch. exper Path. Pharmacol. 176 63 1934.
- Beer R. Re gen IV and J uen J. Am. Heart J 41 115 1931.
- Belet S. Clinical Disorders of the Heart Beat, Lea and Febiger Philadelphia 1963.
- Bergtzen E. Acta med. scandinav 154 21 1954 a.
- Bergtzen E. Acta med. scandinav 154 91 1954 b.
- Bergtzen E. Acta med scandinav 154 225, 1954 c.
- Bergtzen E. Studies on the Electrocardiogram and Working Capacity Stockholm 1957.
- Buckel G. Plummer H. and Edelstein, H. Arch. mal. coeur 44 61 1951.
- Burr R. St George S. and Friedman M. Proc. Soc. Exper Biol & Med 79 513 1952.
- ✓ Bing R. J. and Daley R. Amer J Med. 10 711 1951.
- ✓ Bing R. J. Margot F. M. Donnan J. J. F. Dr per J. A., Heinbocker R., Daley R. Gerard R. and Gelzer, P. Circulation 2 513 1950.
- Björck, G. Brit. Heart J 9 181, 1947.
- Björck, G. Jackson F. S. and Reelin S. Acta med. scandinav 152 283 1948.
- Bjar A. and Lau H. H. Upsala Ill. Ösch. 33 1 1927.
- Borison, H. L. J. Pharm. & Pharmacol 104 396, 1952.
- Borison H. L. and Wang S. C. Proc. Soc. Exper Biol. & Med. 76:335 1951.
- Brownwald E., Bloodcraft R. D., Goldberg L. J. and Morrow A. G. J Clin. Invest. 40 32, 1961.
- Burnell B. and Hendle J. P. Am. J. Med. 8 640, 1950.
- Büchner F. Klin. Wochenschr 33 1237 1933.
- Büchner F. Arch. exper Path. u. Pharmacol. 176 59 1934.
- Camphell, M. Brit. Heart J 4 131 1942.
- Carnau, P. and Sjöstrand, T. Acta med. scandinav 146 191 1953.
- ✓ Carlsson L. A. and Fernow B. Acta med. scandinav 164 39 1959.
- Cattell McKern and Gold H. J. Pharmacol. & Exper Therap. 62 116, 1938.
- Chen S. Y. and Daweide F. R. Chinese J Physiol 3 217 1931.
- ✓ Cohen, M. B. New England J Med. 246 225 1952 a.
- ✓ Cohen B. M. New England J Med. 246 234 1952 b.
- Cohn A. E. and Frazer F. R. J Pharm. & Pharmacol. 5 512, 1913.
- Cohn A. E. and Stewart H. J. J Clin. Invest. 6 53 1928.
- Cotte de V. M. and Stopp, Ph. E. Am. J Physiol. 192 114 1938.
- Dawson, W. H. Barner A. R. and Essie H. E. Am. Heart J. 25 668 1943 a.
- Dawson, W. H. Barner A. R. and Essie, H. E., Am Heart J 25 648, 1943 b.
- DeGraff A. New York J Med. 46 1800 1946.
- Dock W. and Tawster M. L. J Clin. Invest. 8 467 1930.
- ✓ Drisdale D. T. Yasougi Y. Z., Michelson R. J. Scholtz M. and Lu ger M., Am. J. Cardiol. 4 88, 1959.

- Dönhardt A* *Zschr Kreislaufforsch*, 40: 548 1951
- Eichna L W* *Taube H* and *DeGruff A J* *Pharmacol. & Exper Therap.* 78 22 1943
- Erickson E W* and *Fahr G E*. *Am. Heart J* 29 318 1945
- Fuker R A* *Statistical Methods for Research Workers* Oliver and Boyd Edinburgh, 1950.
- Flaxman N* *Am. J. M. Sc.* 216 179 1948.
- Friedberg Ch. A.* and *Zager A* *Circulation* 29 633 1961
- Friedman M* *Bae R.*, *Byers S O* and *Blend C* *Circulation* 2 749 1950.
- Gardberg M* *Clinical Electrocardiography* Harper & Brothers New York 1957
- Gardberg M* and *Ashman R*. *Arch. Int. Med.* 26 849 1943
- Griger A J* *Blaney L F* and *Druckmüller W H* *Am. Heart J* 22 230, 1941
- Gückrist A R* *Scottish M J* 3 35 1958.
- Gold H* *Fed. Proc.* 3 72 1944
- Gold H* *Castell M* *Awst N* and *Kramer M J* *Pharmacol. & Exper Therap.* 75 212 1941
- Gold H* *Awst A T* *Castell M* *Acheson and Travell J J* *L. M. A.* 119 928 1942.
- Gold H* *Castell M* *Ache Modell W* *Germer T* and *Gueve R.* *J Pharm. & Pharmacol.* 98 357 1950.
- Gold H* *Greiner T* *Castell M* *Modell W* *Gluck J* *Marish R* *Alakes S* *Hadio D* *Robertson R* *Warshaw L* *Otto H* *Awst A* and *Kramer M* *Am. J Med.* 13 124 1952.
- Gold H* *Awst V T.* *Otto H* and *Fox T J* *Clin. Invest.* 18 429 1939
- Gold H* *Castell M* *Acheson Awst V T* and *Kramer M J* *Pharmacol. & Therap.* 75 212 1941
- Goldberger E.* *Unipolar Lead Electrocardiography and Vectorcardiography* Lea & Febiger Philadelphia, 1955
- Gremels H* *Arch. exper. Path. u. Pharmacol.* 179 360 1935
- Grenn A. E.* *Some Supplementary leads in Clinical Electrocardiography* - Stockholm 1948.
- Harvey R M* *Ferrer I* *Carthcart R T* and *Alexander J A.* *Circulation* 4 366 1951
- Hatcher R. A* and *Weiss S* *Arch. Int. Med.* 29 690, 1922
- Hedlund S.* *Cardiologia* 20 suppl ad, 1932.
- Helander S* and *Östman J* *Svenska Läk. tidn.* 57: 3706 1960
- Hodley O F* *Ann. Int. Med* 18 154 1943
- Hellström R.* *Acta med. scandinav* Suppl. 371 1961
- Herrman G R.* *Decherd C M* and *McAlley W F J A M A* 118 760 1944
- Heymans J F* and *Heymans C J* *Pharm. & Pharmacol.* 79 203 1926.
- Holmgren A* *Scandinavian J Clin. & Lab. Invest.* 8 suppl. 24 1956.
- Holmgren A.* and *Alfsson A. H* *Scandinavian J Clin. & Lab. Invest* 6 137 1954
- Holmgren A* and *Persson B* *Scandinavian J Clin. & Lab. Invest.* 12 224 1960.
- Holmgren A* and *Strandell, T* *Acta med. scandinav* 169 57 1961
- Holmgren A* and *Stäm G* *Acta med. scandinav* 163 185 1959
- Holmgren A* *Jonsson B* *Leander Maj* *Lindholm H* *Sjöstrand T* and *Ström G* *Acta med scandinav* 158 413 1957
- Holmgren A* *Jonsson B* *Leander Maj* *Lindholm H* *Sjöstrand T* and *Ström G.* *Acta med. scandinav* 165 239 1959
- Jönvall S* *Acta radiol.* 20 325 1939
- Jönvall S* and *Sjöstrand T* *Acta physiol. scandinav* 3 49 1941
- Kate L. N* *Redbard S.* *Friend M* *Rotterdam H J* *Pharmacol. & Exper Therap.* 62 1 1938
- Kemp T* and *Nielsen A* *Statistik for medicinske Munksgaard Copenhagen*, 1939
- Kjellberg, S R* *Lönroth H* and *Rudhe U* *Acta radiol.* 35 413 1951
- Kjellberg S R.* *Rudhe U* and *Spist and T* *Acta radiol.* III 115 1949
- Kjellberg, S R.* *Lönroth H* *Rudhe U* and *Sjöstrand T* *Acta med. scandinav* 140 446 1951
- Kosibi E H* *Cardiologia* 17 79 1930.
- LaDue J* *Proc. Soc. Exper Biol. Med.* 46 631 1941
- Lagerlöf H* and *Werkö L.* *Acta cardiol.* 4 1 1949
- Landgren J* *Personal communication* 1964
- Larsen K. H* and *Nielsen N A* *Hospitabrid.* 37 919 1936.

- Larson, K. H., Veith & F. and Nielsen V  
A. Am. Heart J 13 163 1937
- Larson H and Kjellberg, S. R. Acta radiol.  
29: 159 1948.
- Lepeschkin, E. Moderna Electrocardiography  
Williams and Wilkins, Baltimore 1951
- Lepeschkin E. and Seronius B.: Circulation  
6 378 1952.
- Leffers E. B. New England J Med. 248 316,  
1953
- Lery R. L., Brown, H. G. and Williams N  
E. Am. Heart J 19 639 1940.
- Liebow I M and Fell, H. Am. Heart J  
22 685 1941
- Liljestrand G and Nylin G. Acta physiol.  
scandinav 1 328 1941
- Linderholm H and Stenell T. Acta med.  
scandinav 182 247 1958.
- Livroth K. Acta med. scandinav Suppl. 524  
1957
- Lloyd-Thames, H. G. Brit. Heart J 23 361  
1961
- Low B and Levine S. A. New England J  
Med. 250 771 1954.
- Lutz, D. Arch. Int. Med. 81 251 1924.
- Lutz, D. The Clinical Use of Digitalis.  
Baillière Tindall & Cox, London, 1936.
- McMichael, J. and Sharpey-Schaefer E. P  
Quart. J. Med. 13 123 1944.
- McMillan, T. and Bollet S. "Effects of Digitalis on the Electrocardiogram" Walshaw D  
Steed Diagnosis and treatment of cardiovascular disease. F. A. Davis Company Philadelphia 1952
- Mitchel D. Deutsches Arch. Klin. Med.  
201 17 1954.
- Moss G. K. and Mindele R. Circulation  
4 729 1951
- Narlsen, N. A. and Thier M. Am. Heart J  
17 513, 1919
- Nordenfjeld O. Acta med. scandinav Suppl.  
19 1941
- Nordström-Öhrberg, G. Medicinska riksstämman 1958.
- Nordström-Öhrberg G. Medicinska riksstämman 1959
- Nordström-Öhrberg, G. Unpublished observation, 1962.
- Nylin, G. Scandnav Arch. Physiol. 69 237  
1954
- Ohlta G. T.: J. Am. Geriatrics Soc. 5 163  
1957
- Ohlta, G. T. Kelley F.E., Tulse P. J. Smith  
L. B. and Gelling E. M. Circulation 7: 161  
1953
- Pard H. E. B. J. A. M. A. 81 106, 1925
- Pick A. Circulation 15: 603 1957
- Rabbe H and Ström O. Acta obst. et gynec.  
scandinav 37 312, 1958.
- Rodman, T., Gorceyca, C. A. and Foster R.  
H. Ann. Int. Med. 55 620 1961
- Rown I R. In Gendberg M. Clinical Electrocardiography Hoeber Harper New York  
1957
- Ross J. Braunwald E. and Waldhausen J. A.  
J. Clin. Invest. 39 937 1960.
- Ross J. Waldhausen J. A. and Braunwald E.  
J. Clin. Invest. 39: 930, 1960.
- Rothlis E. and Burker R. Ergeb. inn. Med.  
u. Kinderh. 5 457 1954.
- Routler D and P. de, V. Arch. mal. coeur  
28 800, 1935.
- Ruskin A. and Decker, O. Proc. Soc. Exper.  
Biol. & Med. 68 463 1948.
- Sagall, E. L. and Wolff L. New England J  
Med. 240: 276 1949
- Sandberg L. Nord. med. 56 1718, 1957
- Sandberg, L. Acta med. scandinav Suppl. 365  
1961
- Sandberg, A. A., Scherlis L., Grisham A. and  
Werner J. Circulation 2 921 1950.
- Schröder G., Melander R., Larsson E.  
and Werkö L. Clin. Pharmacol. Therap.  
3 425 1962.
- Selzer A., Hallgren, H. A. Ebnerth C. L.,  
Bradley H. W. and Stone A. O. Brit. Heart  
J 21 335 1959.
- Sinason G. Am. Heart J 64 401 1962.
- Sjöstrand, T. Acta med. scandinav Suppl.  
196 687 1947
- Sjöstrand T. Acta physiol. scandinav 11 211  
1948
- Sjöstrand, T. Acta med. scandinav 138 191  
1950a.
- Sjöstrand T. Acta med. scandinav 138 201  
1950b.
- Sjöstrand T. Acta physiol. scandinav 24 247  
1951
- Sjöstrand T. Verhandl. deutsch. Gesellsch.  
Krebsforsch. 22. Tagung 143 1956

- Dönkerdt A Ztschr Kreislaufforsch. 40 528, 1951
- Eick a L. W. Tanabe H and D G aff A J Pharmacol. & Exper Therap 78 22, 1943
- Erickson E. W. and Fahr G E. Am. Heart J 29 318 1945
- Fisher R A Statistical Methods for Research Workers Oliver and Boyd Edinburgh 1930.
- Flaxman N Am. J. M. Sc. 216 179 1948.
- Friedberg Ch. A. and Zager A Circulation 23 655 1961
- Friedman M Bize R Byers S O and Bland G Circulation 2: 749 1950.
- Gardberg, M Clinical Electrocardiography Harper & Brothers New York 1957
- Gardberg M and Ashman R Arch. Int. Med. 26: 842 1943
- Genger A J Blaney L. F and Dr ckemiller W H Am. Heart J 72 230 1941
- Glückhut A R Scottish M J 9 53 1958.
- Gold H Fed. Proc. 3: 72 1944
- Gold H Cattell M Kunt N and Kramer M J Pharmacol. & Exper Therap. 73 212, 1941
- Gold H Kunt N T Cattell McKeen and Trapell J J A. M. A. 119 928 1942.
- Gold H Cattell McKeen Modell W Gerner T and Gutwera R. J Pharm. & Pharmacol. 98 337 1950
- Gold H Greiner T Cattell M Modell W Gluck J Marsh R Mathes S Hadso D Robertsson R Wavshaw L. Otto H Kunt N and Kramer M Am. J Med. 13 124 1952.
- Gold H., Kunt N T Otto H and Fox T J Clin. Invest. 18 429 1939
- Gold H Cattell McKeen Kunt N T and Kramer M J Pharmacol. & Therap. 73 212 1941
- Goldberger E Unipolar Lead Electrocardiography and Vectorcardiography Lea & Febiger Philadelphia, 1953
- Gremels H Arch. exper Path. u. Pharmacol. 179 360 1955
- Green K. E. Some Supplementary leads in Clinical Electrocardiography Stockholm 1948.
- Harvey R M Ferrer I Carthcart R. T and Alexander J A Circulation 4 366 1951
- Hatcher R. A and Weiss S Arch. Int. Med. 29 690 1922.
- Hedlund E Cardiologia 20 suppl. ad. 1932
- Holander S and Ostman J Svenska Läk. tidn. 57 3706 1960.
- Hedley O F Ann. Int. Med. 18 154 1943.
- Holmér R Acta med. scandinav Suppl. 371 1961
- Herrman G R Dechard O M and McKinlay W F J A. M. A 18 760 1944
- Heymans J F and Heymans C J Pharm. & Pharmacol. 29 203 1926
- Holmgren A Scandnav J Clin. & Lab. Invest. 8 suppl. 24 1956.
- Holmgren A and Mattson A. H Scandnav J Clin. & Lab. Invest 6 137 1954
- Holmgren A. and Pernow B. Scandnav J Clin. & Lab. Invest. 12 224 1960
- Holmgren A. and Strandell T Acta med. scandinav 169 57 1961
- Holmgren A and Sten G Acta med. scandinav 163 185 1959
- Holmgren A Jonsson B Leander Maj Linderholm H Sjöstrand T and Ström G Acta med. scandinav 158 413 1957
- Holmgren A., Jonsson B. Leander M., Linderholm H Sjöstrand T and Sten G. Acta med. scandinav 165 259 1959
- Jonell S Acta radiol. 20 325 1939
- Jonell S and Sjöstrand T Acta physiol. scandinav 3 49 1941
- Katz L. N Rodbard S Friend M Rotterman W J Pharmacol. & Exper Therap. 62 1 1938
- Kemp T and Nielsen A Statistik for medicinere Årshjælper København, 1939
- Kjellberg S R., Lönnroth H and Rudhe U Acta radiol. 35 413 1951
- Kjellberg S R. R. dhe U and Sjöstrand T Acta radiol. 31 115 1949
- Kjellberg, S R Lönnroth H Rudhe U and Sjöstra d T Acta med. scandina 140 446 1951
- Koelbing H Cardiologia 17 79 1950.
- LaDue J Proc. Soc. Exper Biol Med. 46 651 1941
- Lagerlöf H and Werkö L. Acta radiol. 4 1 1949
- Landgren J Personal communication 1964
- Larss A. H and Nielsen V A Hospitaltid. 37 949 1936.

## SUMMARY AND CONCLUSIONS

The aim of this study was to investigate the effect of cardioactive glycosides on the circulatory response and on the ECG reaction during muscular exercise.

A total of 92 digitalizations with lanatoside C, digitoxin and digoxin was performed on 64 healthy volunteers.

After administration of cardioactive glycosides considerable reductions of heart rate at rest were observable in individual cases, though none in some. The differences are, however, not significant.

The orthostatic pulse reaction, i.e. the difference between heart rate in recumbent and standing position was considerably and highly significantly greater after administration of lanatoside C, even in small doses.

This reaction became still more marked after lanatoside C if the doses were toxic in the meaning that subjective discomfort or other symptoms appeared. No similar effect was found after digitoxin or digoxin, however not even after toxic doses.

Intravenous lanatoside C caused no significant change of heart rate during or after exercise.

After oral administration of digitoxin the heart rate was rather lower during exercise at low loads, whereas there was no significant difference at higher loads. The post-exercise heart rate was unchanged before and after digitalization. Ventilation, oxygen uptake and lactic acid concentration were unchanged by digitalization.

Thus digitalis did not affect the calculated physical working capacity at maximal heart rate, nor the working capacity heart rate of 170 beats/min.

It also appeared from these investigations

that the degree of orthostatism in the standing position does not influence the circulatory response during sitting exercise.

The heart volume in prone position and the total amount of haemoglobin were not changed after digitalization. On the other hand the standing heart rate declined slightly both after lanatoside C and after digitoxin.

In conclusion, these studies have shown that the digitalis glycosides do not exert such a haemodynamic effect on healthy subjects as to influence the circulatory response to an exercise test at submaximal loads to a significant degree.

The ECG changes in conjunction with digitalizations are analysed in Part II.

The frequency of premature beats ascribable to digitalization was extremely low at rest, standing, and during and after exercise. The A-V conduction was slightly prolonged after digitalization, in particular immediately after exercise. During exercise, on the other hand, there was no difference in A-V time before and after digitalization.

The most common changes were observed in the ST-T region and are described as 11 types.

Digitalis-induced ST-T changes were recorded comparatively seldom at rest and were generally slight. In the standing position, on the other hand, they were much more common. In the total of 92 tests digitalis glycosides provoked changes in the ST-T region during exercise in 89 per cent of the subjects. But immediately after exercise these changes had receded and only 39 per cent of the subjects exhibited them. Three minutes after the end of exercise the changes were somewhat accentuated,



- Stewart H J and Cohn A. E. J Clin. Invest. 11 917 1932.
- Stewart H J and Watson R. F. Am. Heart J 15 604 1938.
- Stoll A. J Pharm. & Pharmacol. 1 849 1949
- Stout H and Katz L. N. Am. Heart J 10 546 1934
- Ström G. Acta physiol. scandinav 17 440 1949
- Tandowsky R. M. Am. Heart J 4 472 1942.
- Tandowsky R. M. Anderson N and Vandersenter J K 28: 298 1944.
- Tillakos M. Brit. Heart J 15 95 1953
- Wahlund H. Acta med. scandinav. Suppl. 21. 1948.
- Wallon R. P., Leary J S and Jones H P. J Pharm. & Pharmacol. 98 346 1950.
- Wadd A. M and Blair H A. J Pharmacol. & Exper Therap 90 211 1947
- Wiegria R. Pharmacol. Rev 3 197 1951
- Wierks L. Eliash H. Thomsen B and Larnaukas E. Cardiologia 33 161 1958.
- Wiggers C J and Stimson B. J Pharm. & Pharmacol. 30 251 1927
- Wiklander O. Acta chir scandinav. Suppl. 208 1956
- Williams H Jr. Zohman L. R. and Ratner A C. J Appl. Physiol. 13 417 1958.
- Withering, W. An Account of the Foxglove and Some of its Medical Uses with Practical Remarks on Dropsy and other Diseases. G. G J and J Robinson, London, 1783 Reprinted in Medical Classics, 2 305 1937
- Wood P. Diseases of the heart and circulation. Eyre and Spottiswoode, London 1956
- Woodbury L. A. and Heckl H H. Circulation 6: 172, 1952.
- Wright S. E. The metabolism of cardiac glucosides. Charles C. Thomas Publisher 1960.
- Yuletic I and Isancic R. Arch. mal. coeur 45: 853 1952.
- Yule P N G., Lovejoy F W, Huijck B. Howell M M, Joss H A, Ten ey S M, Haroutunian L. M and Evans H D. Am. J M. Sc. 224 146 1952.
- Zwilling L. Med. Klin. 31 977 1935
- Åstrand Irma. Acta physiol. scandinav. Suppl. 169 1960.
- Åstrand P O. Experimental studies of physical working capacity in relation to sex and age. Munksgaard Copenhagen, 1952.

## ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Associate Professor Bengt Pernow head of the Department of Clinical Physiology at Serafimerlasarettet, for his stimulating advice and interest and his very generous support during the course of this work. My thanks are due also to Professor Gunnar Björck for valuable discussions and for his profitable advice concerning the preparation of the manuscript.

I also wish to thank Professor Gunnar Ström, Uppsala, during whose time as head of the Department of Clinical Physiology at Serafimerlasarettet this work was started, for his encouragement during

the entire course of the study and for his advice and constructive criticism.

My special thanks are due to Nurse Ingrid Ollén for her assistance, to Mrs. Agnes Roschild and Mrs. Berith Hultenberg for help with the laboratory work, to Mr. Torkel Gårding who did most of the statistical analysis, and to Mr. John Hogg for his translation into English. Also to Mrs. Birgit Hambræus who, throughout the whole study has given me skilled, valuable and always encouraging assistance in typing.

The investigation was supported by grants from the Swedish National Association against Heart and Chest Diseases.

and 48 per cent of the subjects now shown showed ST T changes.

The changes during the post-exercise period were not more pronounced than during exercise.

If the ST T region was unchanged during exercise, no ST T changes occurred either in digitalized subjects after exercise.

There was no difference as regards the effect of lanatoside C and digitoxin on the ST T region. Nor does this study show that digoxin has a different effect on the ECG from that of the two other glucosides.

One may thus say in conclusion that digitalis glucosides may cause several types of ST T changes, and that in the exercise ECG some of these changes are virtually undistinguishable from those reported as typical of coronary insufficiency. The appearance of ST T changes already in the

standing position, and the quick restitution of the digitalized subjects after exercise, however may be of some assistance for the differential diagnosis. But as shown in this study there are occasions when the ST T region is not changed in the standing position and does not become normalized after the end of exercise.

It must therefore be emphasized that, to avoid false positive responses to exercise tests, it is important that, in the case of a compound with a prolonged effect such as digitoxin, a sufficiently long period (at least six weeks) should have elapsed since the administration if the intention is to assess from the ECG reaction whether coronary insufficiency is present or not.

In this investigation the effect of digitalis glucosides was studied on subjects with healthy hearts. It is conceivable that in cases of at least badly diseased hearts the effect may be different.









# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 419

## A STUDY OF BRONCHIAL ASTHMA

*with special reference  
to its long term consequences  
for respiratory and circulatory  
function and socio-medical  
condition*

By  
LARS IRNELL



# ACTA MEDICA SCANDINAVICA

has been published since 1910 as a continuation of *Nordiskt Medicinskt Arkiv* founded in 1869 by Axel Key. The first volume of *Acta Medica Scandinavica* is therefore numbered LII (52).

The chief editors have been Axel Key 1869—1900 C. G. Santesson 1901—1915 I. Holmgren 1916—1957 and Birger Strandell 1958 to date.

*Acta Medica Scandinavica* publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board. Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the Editor at the address below.

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that if accepted, it will not be republished in any other journal in the same or similar form without the Editor's written consent.

Papers will be published in English, French or German at the authors' choice, followed by a summary in English.

The manuscripts shall be in final form as submitted. They shall be type written with double spacing and broad left hand margin.

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author. No paper shall exceed 24 printed pages.

## Subscription

The annual subscription to the journal covering two volumes, each of 6 numbers, is 140 Sw. crowns or U.S. \$ 27.25 including postage in the Scandinavian countries and in Holland 120 Sw. crowns.

*Address for subscriptions and all communications*

ACTA MEDICA SCANDINAVICA

P. O. Box 2052 Stockholm 2

---

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number.

From the University Hospital, Uppsala, Sweden

Department of Internal Medicine

Head Professor E. A. L. Uppmark, M.D.

Department of Clinical Physiology

Head Professor C. Ström, M.D.

Department of Social Medicine

Head Professor R. Berfemstam, M.D.

# A STUDY OF BRONCHIAL ASTHMA

*with special reference  
to its long term consequences  
for respiratory and circulatory  
function and socio-medical  
condition*

By

IARS IRNELL

Statistical advice: Gunnar Ekblad, Ph. D.

UPPSALA 1984

# ACTA MEDICA SCANDINAVICA

has been published since 1910 as a continuation of *Nordiskt Medicinskt Arkiv* founded in 1869 by Axel Key. The first volume of *Acta Medica Scandinavica* is therefore numbered LII (52).

The chief editors have been Axel Key 1869—1900, C. G. Santesson 1901—1915, I. Holmgren 1916—1957 and Birger Strandell 1958 to date.

*Acta Medica Scandinavica* publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board. Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the Editor at the address below.

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that if accepted, it will not be republished in any other journal in the same or similar form without the Editor's written consent.

Papers will be published in English, French or German at the authors' choice, followed by a summary in English.

The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left hand margin.

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author. No paper shall exceed 24 printed pages.

## Subscription

The annual subscription to the journal, covering two volumes, each of 6 numbers, is 140 Sw. crowns or U.S. \$ 27.25 including postage in the Scandinavian countries and in Holland 120 Sw. crowns.

*Address for subscriptions and all communications*

ACTA MEDICA SCANDINAVICA

P. O. Box 2052 Stockholm 2

---

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number.

# CONTENTS

I. Introduction and aim of the investigation	5
II. Definitions	7
III. Survey of literature	9
IV. Material	15
Principles used in the collection of the material	15
Evaluation of the composition and completeness of the material	19
V. Methods	24
Assessment of clinical course with respect to the estimated degree of severity of the bronchial asthma	24
Analysis of circulatory and respiratory function	25
Principles of classification in the treatment of socio-medical problems	30
VI. Description of clinical course with regard to the estimated degree of severity of the bronchial asthma	33
VII. Respiratory and circulatory function in relation to the clinical course and estimated degree of severity of the bronchial asthma	44
Lung function studies	40
Studies of physical work capacity	64
Hemodynamic studies	76
Discussion	93
Summary	113
VIII. Socio-medical aspects	117
Mortality	117
Invalid pension	118
Change of residence	119
General education and family data	122
Wage-earning	126
Income level	133
Housing standard and size	135
Smoking and alcohol consumption	141
Discussion	143
Summary	152
IX. General summary	153
Appendix 1. Statistical methods	158
Appendix 2. Case reports of patients with chronic disease in addition to the bronchial asthma	169
Appendix 3. Key to the ward case record	165
References	166
Acknowledgements	171

Also published as  
SUPPLEMENT  
of ACTA MEDICA SCANDINAVICA

*Translated by Maud Marsden*

# CONTENTS

I. Introduction and aim of the investigation	5
II. Definitions	7
III. Survey of literature	9
IV. Material	18
Principles used in the collection of the material	18
Evaluation of the composition and completeness of the material	19
V. Methods	21
Assessment of clinical course with respect to the estimated degree of severity of the bronchial asthma	24
Analysis of circulatory and respiratory function	26
Principles of classification in the treatment of socio-medical problems	30
VI. Description of clinical course with regard to the estimated degree of severity of the bronchial asthma	33
VII. Respiratory and circulatory function in relation to the clinical course and estimated degree of severity of the bronchial asthma	44
Lung function studies	40
Studies of physical work capacity	64
Hemodynamic studies	76
Discussion	98
Summary	113
VIII. Socio-medical aspects	117
Mortality	117
Valid pension	118
Change of residence	119
General education and employment	122
Wage-earning	126
Income level	133
Housing standard and size	135
Smoking and alcohol consumption	141
Discussion	143
Summary	152
IX. General summary	156
Appendix 1. Statistical methods	158
Appendix 2. Case reports of patients with chronic disease in addition to the bronchial asthma	160
Appendix 3. Key to the ward case records	163
References	166
Acknowledgements	171

Also published as  
SUPPLEMENT  
of ACTA MEDICA SCANDINAVICA

*Translated by Maud Marsden*

# I INTRODUCTION AND AIM OF THE INVESTIGATION

Those treating patients for bronchial asthma, often in a serious condition, may ask How does a disease such as this affect the individual in the long view? Many of the patients appear in hospital time and time again, often in an apparently serious condition. They may become 'regular customers' both in the out patients department and on the wards, and from their histories of frequent, more or less pronounced symptoms, it may be assumed that for many of them the disease has indeed influenced their lives.

Others belong to the group of patients who, although they may also seek hospital treatment on one or more occasions with severe asthma, are then seldom or never seen there again. Of their subsequent progress little is known after their few hospital visits there is often no further information either on their ward case records or their out patient cards. Have they recovered? Or has the asthma changed in character so that the acute attacks necessitating hospital treatment have subsided? Have special efforts on the part of the patients doctor or community produced a change for the better

Questions of this kind are of especial

interest to the clinician, and it was therefore considered justifiable to study this matter further

---

It is difficult to obtain adequate information on the prognosis in adult patients with bronchial asthma, for several reasons. The main difficulty is caused by the confusion in the literature as to the definition of the terms bronchial asthma chronic bronchitis and pulmonary emphysema. Each of these terms sometimes appear to be used by different clinicians to describe the condition in one and the same patient.

The large spontaneous variations and often very irregular course of this disease have probably to a great extent discouraged detailed analyses of its influence on adult persons. The investigations described in the literature have usually comprised small series of cases, and as a rule attention has been paid to only one or a small number of those factors which when combined contribute towards a summarizing assessment of the prognosis of the disease. In the different investigations reported attempt has often been made to correlate the prognosis of the





## II DEFINITIONS

Considerable attempt has been made during recent years to produce exact clinical definitions and classifications of bronchial asthma, chronic bronchitis and pulmonary emphysema—summarized in the term chronic obstructive pulmonary diseases. Different authors have tended to define these conditions in different ways. The lack of generally accepted definitions and classifications has obviously delayed clinical research in this field, and has also decreased the possibility of utilizing to its full extent the experience gained, of evaluating results and making comparative studies. It is clear that further knowledge of these diseases is required for the establishment of more generally accepted definitions.

As pointed out by the American Thoracic Society (ATS) Committee on Diagnostic Standards for Nontuberculous Respiratory Diseases (1962) a disease may be defined according to its etiology, the characteristic morphological changes which it causes, the functional disturbances it produces or its clinical manifestations. In chronic bronchitis and bronchial asthma there appears to be no individual manifestation or combination of criteria which permits an exact definition. The ATS

further states that the definitions of these conditions are therefore based essentially on the description of clinical manifestations which in themselves are not pathognomonic. In pulmonary emphysema on the other hand, the anatomical changes are sufficiently specific to allow with the use of anatomical terminology a definition of this condition.

In the following, definitions of bronchial asthma, chronic bronchitis and pulmonary emphysema, as used in the present investigation, will be given. The recommendations published by the ATS were followed to a large extent. The descriptions in quotation marks are cited from these recommendations.

**Chronic bronchitis** Chronic bronchitis is a clinical disorder characterized by excessive mucous secretion in the bronchial tree. It is manifested by chronic or recurrent productive cough.

The expectoration should not be due to localized broncho-pulmonary disease or cardiac disorders. I have defined the words chronic or recurrent as present on practically all days during at least three months in each of more than two successive years.

**Bronchial asthma** Asthma is a

asthma with certain forms of therapy or certain types of the disease, less attention being paid to general assessments of functional sequelae.

It may be assumed that during the early stages of the disease the bronchial obstruction and its sequelae are only temporary. On the other hand it may be expected that repeated attacks or prolonged periods of difficulty in expiration may lead to irreversible changes especially pulmonary but also cardiac. Similarly socio-medical functional impairment might also be expected. *The aim of the present investigation was to describe the clinical course of the disease in a series of adult patients with bronchial asthma of varying duration and severity selected according to certain norms for the purpose of studying—in relation to the estimated severity and course—the long-term effect of the disease both on the respiratory and circulatory functions and on the social situation of the patients*

The effect of the disease on the re-

spiratory and circulatory functions was assessed by studying the ventilation capacity lung volumes and physical work capacity and also hemodynamic data obtained by means of right heart catheterization. The results obtained were compared with corresponding data from control materials and were also correlated to the estimated degree of severity of the disease.

The effect of the disease on social adaptation was studied by assessing the status of the patients in this series as compared with control materials, using certain indicators. The social situation of these patients was studied for the year 1960 and comparisons were made with the situation for the corresponding total population as indicated by the housing and population census for that year and other available control materials. Among the indicators studied, the main items were family data general education, changes of residence, adaptability with regard to life at work, income, and housing conditions

## II DEFINITIONS

Considerable attempt has been made during recent years to produce exact clinical definitions and classifications of bronchial asthma, chronic bronchitis and pulmonary emphysema—summarized in the term chronic obstructive pulmonary diseases. Different authors have tended to define these conditions in different ways. The lack of generally accepted definitions and classifications has obviously delayed clinical research in this field, and has also decreased the possibility of utilizing to its full extent the experience gained, of evaluating results and making comparative studies. It is clear that further knowledge of these diseases is required for the establishment of more generally accepted definitions.

As pointed out by the American Thoracic Society (ATS) Committee on Diagnostic Standards for Nontuberculous Respiratory Diseases (1962) a disease may be defined according to its etiology, the characteristic morphological changes which it causes, the functional disturbances it produces or its clinical manifestations. In chronic bronchitis and bronchial asthma there appears to be no individual manifestation or combination of criteria which permits an exact definition. The ATS

further states that the definitions of these conditions are therefore based essentially on the description of clinical manifestations which in themselves are not pathognomonic. In pulmonary emphysema, on the other hand, the anatomical changes are sufficiently specific to allow with the use of anatomical terminology a definition of this condition.

In the following, definitions of bronchial asthma, chronic bronchitis and pulmonary emphysema as used in the present investigation, will be given. The recommendations published by the ATS were followed to a large extent. The descriptions in quotation marks are cited from these recommendations.

*Chronic bronchitis* Chronic bronchitis is a clinical disorder characterized by excessive mucous secretion in the bronchial tree. It is manifested by chronic or recurrent productive cough.

The expectation should not be due to localized broncho-pulmonary disease or cardiac disorders. I have defined the words "chronic" or "recurrent" as present on practically all days during at least three months in each of more than two successive years.

*Bronchial asthma* Asthma is a

disease characterized by an increased responsiveness of the trachea and bronchi to various stimuli and manifested by a widespread narrowing of the airways that changes in severity either spontaneously or as a result of therapy.

In the present study the term bronchial asthma is not used if the criteria of chronic or recurrent cough are considered to be fulfilled in accordance with the conditions stated in the definition of chronic bronchitis. In agreement with the ATS I consider that "the term asthma is not appropriate for the bronchial narrowing which results solely from destructive diseases of the lung e.g. pulmonary emphysema or from cardiovascular disorders".

The characteristics of the clinical manifestations of bronchial asthma found in the present study are in concordance with the description made by the ATS where bronchial asthma is said to be clinically char-

acterized by episodes of dyspnea, cough and wheezing. These episodes may be transitory and subside within one or two days, or they may be prolonged. Some episodes may be extremely severe and result in death. During these episodes physical examination may reveal prolongation of expiration, an overexpanded thorax and hyperresonance to percussion. On auscultation, high pitched wheezing sounds are heard throughout the entire thorax. They are audible both in inspiration and expiration but are more prominent in expiration.

Characteristic of the disease is its intermittent course with symptomatic periods of varying lengths between more or less symptom-free intervals. There is usually an allergic background, but this cannot always be shown.

*Pulmonary emphysema.* Emphysema is an anatomic alteration of the lung characterized by abnormal enlargement of the air spaces distal to the terminal non-respiratory bronchiole accompanied by destructive changes of the alveolar walls.

### III SURVEY OF LITERATURE

Owing to the lack of generally accepted definitions of chronic obstructive pulmonary diseases, it is difficult in studying the literature to form an idea of the sequelae of bronchial asthma. When this condition is defined, which is fairly seldom, the definitions vary often depending on the discipline or country in which the author is active. Clear delimitation from other chronic obstructive lung diseases is not often made.

On studying the literature attempt was made to obtain data on the following

- 1 Results of clinical follow up examinations of patients with chronic obstructive pulmonary disease.
- 2 Result of studies aimed at finding out whether the cardio-respiratory functional changes in bronchial asthma differ from those in other chronic obstructive pulmonary diseases.

Special attention was paid to the effect on respiratory function, which can be assessed by means of determinations of the lung volume and ventilation capacity (2a) and circulatory function, which can be assessed by means of flow and pressure determinations made

during right heart catheterization (2b)

- 3 Socio-medical aspects of patients with chronic obstructive pulmonary disease (3a)

No reports of systematic investigations treating essential indicators of the sequelae of such diseases from the point of view of social adaptation were found. It was therefore considered justified, for the purpose of discussing the social consequences of this disease in comparison with other chronic morbid conditions, to make a brief complementary review of the literature concerning socio-medical sequelae of chronic diseases in general concentrating on Swedish investigations (3b)

#### *Clinical follow-up studies*

Ogilvie (1962) reported a study on 1000 unselected patients with bronchial asthma who were followed up for an average period of 11 years. Bronchial asthma was defined as a condition characterized by recurrent bouts of wheezing, mainly or entirely expiratory with an associated variable degree of difficulty in breathing and a expiratory obstruction. If the asthma had symptom free intervals it was described as intermittent and if wheezing and dyspnoea persisted it was called continuous. Chronic

bronchitis was defined as a condition characterized by cough and sputum persistent throughout the winter or throughout the year with a minimum duration of two years the absence of other diseases of the lungs is implicit. It may be stressed that many of the 1000 patients in this series had or had had chronic bronchitis besides their asthma.

This author found that the prognosis in the intermittent form of asthma was more favourable than in the continuous type. The outlook was also found to be more favourable in those cases where the disease was manifested before the age of 15 years. The risk that the asthma would gradually become complicated by bronchitis was greater for those with the continuous type. At the follow up examinations the general condition of the asthmatic patients with bronchitis appeared to be much poorer than that of the patients without this complication. The author claimed to have shown that treatment with specific hyposensitization had no noteworthy effect on the long term prognosis.

Reid and Fairbairn (1938) reviewed the sickness records of 517 postmen who had retired prematurely because of chronic bronchitis, and noted their death rate and causes of death after intervals varying from three to seven years. The ratio of observed to expected deaths was 4.5:1.

Medvei and Oswald (1963) reported a five-year follow up study of 312 bronchitic patients. Approximately 75% of the patients were men of ages 45-64 years. The criteria for inclusion in the study were cough and sputum, not necessarily continuous for at least a year which could not be attributed to any other important primary or precipitating disease of the respiratory cardiovascular or other systems. The ratio of observed to expected deaths of the males was 4.2:1 this declined with increasing age. The authors stated that this ratio showed good agreement with that reported earlier by Reid and Fairbairn (1938). It was pointed out, further, that other results from these two investigations also showed strikingly high degree of concordance. For example in 63% of the patients who died during the observational period, the cause of death was respiratory insufficiency. The corresponding

figure in the investigation of Reid and Fairbairn was 58%. In both studies circulatory insufficiency was the cause of death in 20% of the cases. In Medvei and Oswald's material 90 patients (31.5%) died during the five year period. It was stated that in all of the 5 patients in whom the cause of death was respiratory insufficiency chronic bronchitis and pulmonary emphysema appeared to be the underlying cause.

With regard to the subsequent development of asthma manifested at an early age, the pediatric literature contains much information, and reference may be made to the reviews published by Kræpelin (1939) Aas (1963) and Rysling (1963).

### Respiratory function

As pointed out by Gaensler (1962) until the end of the 1950's there was practically no sub-division of the chronic obstructive pulmonary diseases in the reports of physiological data from these patients. It was at this time that more intensive efforts to define and differentiate between bronchial asthma, chronic bronchitis and pulmonary emphysema were begun.

Three monographs (Stuart-Harris and Hanley 1957; Ogilvie and Newell 1957; Oswald 1958) treating chronic bronchitis and to some extent the functional impairment ascribed to this disease, were published at the end of the 1950's.

The problem of definition and differentiation in this disease group, however, is not yet completely solved. Some light is shed on the question by the fact that Remzell, Eastman, Auchincloss, Gilbert, Elch and Elton (1958) who carried out physiological studies on patients with chronic bronchitis, limited some of these from the final result because their residual volume constituted more than 35% of the total lung capacity a classification that must be regarded as highly arbitrary. Chronic bronchitis was a

fixed condition in which at least 10 attacks of coughing occurred each day for at least six months, provided that there were no signs of other broncho-pulmonary disease. This definition is also obviously arbitrary.

Fletcher, Hugh-Jones, M. Nicol and Pride (1953) studied the occurrence of pulmonary emphysema in chronic bronchitis. These authors found that many of the clinical and physiological changes that are usually regarded as indicating the presence of pulmonary emphysema, only do this in the functional sense i.e. in the sense of hyperinflation. These changes cannot be regarded as evidence of the presence of destructive emphysema. The authors recommend that the word emphysema should be reserved for cases where obvious functional and radiological signs of alveolar tissue destruction are found.

The above investigations are examples of those aimed at studying one of the group of obstructive pulmonary diseases, in these cases chronic bronchitis. Only exceptionally have reports been made of investigations on patients with bronchial asthma who could all or practically all be considered free from chronic bronchitis.

One of these exceptions is the lung function study carried out by Hirschman, Breznick and Segal (1955) on 43 asthma patients of ages 15-72 years, in symptom-free intervals or periods when the symptoms were least pronounced. The vital capacity was found to vary between 81 and 158% and the maximum voluntary ventilation between 11 and 130% in both cases of the predicted normal values. The mean total lung capacity was 123% of the predicted normal value, and the residual quotient varied between 35 and 57% with a mean value of 47. The mean maximum ventilatory volume per second was 3.9 litres.

In several reports, although the title indicates that the results concern only

patients with bronchial asthma mention may be found in the text that the material consists of a mixture of patients with asthma, chronic bronchitis and emphysema. If the functional changes differ in the different types of chronic obstructive pulmonary diseases, an intermixture such as this must obviously result in misleading reports. Because of the confusion in the diagnoses of patients in the earlier studies, it is often difficult when attempting, for example to assess the long term effects of bronchial asthma as such to interpret the results and gain any direct information.

In the earlier literature there are many reports of results from determinations of the lung volume and/or ventilation capacity in emphysema, for example Lundsgaard and Schlerbeck (1932) Hartado, Kaitreider, Gray, Brooks and McCann (1934) Baldwin (1945) Baldwin, Courmand and Dickinson (1949) Bates, Knott and Christie (1956) Beale, Fowler and Comroe (1953) Pugh and Lee (1961) and (1961) Kahan, Aronoff and Plare (1963).

Also of importance in assessing the results from the different investigations is detailed knowledge of the apparatus used in the dynamic lung function tests. The results depend among other things on the amount of resistance to respiration provided by the apparatus. The differences in the methods used, together with the frequent lack of definition of the disease condition described have constituted great barriers to the analysis and assessment of the studies mentioned. These investigations thus provide no definite guidance with regard to the problems of the present study.



bronchitis was defined as a condition characterized by cough and sputum persistent throughout the winter or throughout the year with a minimum duration of two years the absence of other diseases of the lungs is implicit. It may be stressed that many of the 1000 patients in this series had or had had chronic bronchitis besides their asthma.

This author found that the prognosis in the intermittent form of asthma was more favourable than in the continuous type. The outlook was also found to be more favourable in those cases where the disease was manifested before the age of 16 years. The risk that the asthma would gradually become complicated by bronchitis was greater for those with the continuous type. At the follow up examinations the general condition of the asthmatic patients with bronchitis appeared to be much poorer than that of the patients without this complication. The author claimed to have shown that treatment with specific hyposensitization had no noteworthy effect on the long term prognosis.

Reid and Fairbairn (1938) reviewed the sickness records of 517 postmen who had retired prematurely because of chronic bronchitis, and noted their death rate and causes of death after intervals varying from three to seven years. The ratio of observed to expected deaths was 4.5/1.

Medvei and Oswald (1962) reported a five-year follow up study of 312 bronchitic patients. Approximately 75% of the patients were men of ages 45-64 years. The criteria for inclusion in the study were cough and sputum, not necessarily continuous for at least a year which could not be attributed to any other important primary or precipitating disease of the respiratory cardiovascular or other systems. The ratio of observed to expected deaths for the males was 4.2/1 this declined with increasing age. The authors stated that this ratio showed good agreement with that reported earlier by Reid and Fairbairn (1938). It was pointed out, further that other results from these two investigations also showed a strikingly high degree of concordance. For example in 63% of the patients who died during the observation period, the cause of death was respiratory insufficiency. The corresponding

figure in the investigation of Reid and Fairbairn was 58%. In both studies circulatory insufficiency was the cause of death in 20% of the cases. In Medvei and Oswald's material 96 patients (31%) died during the five-year period. It was stated that in all of the 57 patients in whom the cause of death was respiratory insufficiency chronic bronchitis and pulmonary emphysema appeared to be the underlying cause.

With regard to the subsequent development of asthma manifested at an early age, the pediatric literature contains much information, and reference may be made to the reviews published by Kraepelin (1950) Aas (1963) and Rysling (1963).

### *Respiratory function*

As pointed out by Gaensler (1962) until the end of the 1950's there was practically no subdivision of the chronic obstructive pulmonary diseases in the reports of physiological data from these patients. It was at this time that more intensive efforts to define and differentiate between bronchial asthma, chronic bronchitis and pulmonary emphysema were begun.

Three monographs (Stuart Harris and Hanley 1957 Ogilvie and Newell 1957 Oswald 1958) treating chronic bronchitis and to some extent the functional impairment ascribed to this disease, were published at the end of the 1950's.

The problem of definition and differentiation in this disease group, however, is not yet completely solved. Some light is shed on the question by the fact that Renzetti, Eastman, Auchincloss, Gilbert, Elch and Dutton (1958) who carried out physiological studies on patients with chronic bronchitis, eliminated some of these from the final result because their residual volume constituted more than 33% of the total lung capacity. A classification in this case can be regarded as highly arbitrary. Chronic bronchitis was defined

condition be considered later however that owing to the small liability of adequate data this had not been particularly successful. The material was collected with the assistance of a number of general practitioners in different parts of Norway. A questionnaire was sent to those asthma patients of whom the author had received information from these practitioners. Claessen states that the material was made up of just over 1500 patients. The author gives the percentage distribution of different occupations of the male patients living in certain rural districts, and compared them with the occurrence of the respective occupations among the entire rural district population in Norway. The handicraft trades and of fishing and forestry work are well represented among the asthma patients, while there is underrepresentation with regard to industry. Claessen found that 7.3% of the men in the material are completely unfit for work due to their asthma. The average age of these men was 53 years, and the average duration of the asthma 18 years. As for the women were completely unfit for work, their average age was 44 years and the average duration of the asthma 16 years. The study of these figures only men and women of ages 18-70 years were included. Of those who were completely incapacitated, the majority had had their first onset of asthma at an adult age. The duration of the asthma of these patients was longer than in the whole material. The total number of completely disabled asthmatics of ages 18-70 years in Norway was estimated to be at least 1700. In comparison to as mentioned that according to Grim (1931) there were 813 asthmatics in Denmark in 1931 who were registered completely disabled by reason of the disease.

With regard to the non-disabled, in the majority of cases the capacity for work was more or less reduced. This was evidenced in great part by changes in occupation or reduction of working hours. Information on the number of working days lost was only obtained for those married persons, who were able to state the length of those for which they had received sickness benefit because of asthma. For the men, 220 L

together there was an average of 20 days in 1945.

No attempt at definition of the disease and differentiation from chronic bronchitis was made. An investigation of this type based on information from several practicing doctors in different districts, however, presents great difficulties, and an assessment of the exact number of disabled bronchoasthmatic patients is hardly possible. The term disabled was not defined; the patients themselves judged whether or not they were completely fit for work. The comparison between the distribution of the different occupations for the asthmatics and for the whole rural population of Norway had to be made with great care, since the author states, the population in the district investigated did not have the same occupational distribution that the total rural districts.

In an article entitled "Social Importance of Allergic Diseases" Williams (1931) reported his study of the disease mortally in the different social groups in England and Wales at the beginning of the 1930's. The material was divided into 3 social groups. Group I and to some extent group II, which were the highest, had a lower mortality than the other social groups. The author further investigated on the number of sick leave days per 10,000 individuals with asthma and other allergic diseases, respectively, in the population of England and Wales. Some hospital statistics on the incidence of asthma diagnoses were also given - both in total figures and again in relation to other diagnoses. In 22 of approximately 210,000 patients discharged from about 30 large hospitals in England, Scotland and Wales asthma was the main diagnosis. Williams did not define asthma, and the term probably covered all chronic obstructive pulmonary diseases.

Inghe (1948) found a probably increased asthma mortality among the female patients in Stockholm, while there was no corresponding increase for the men. The author assumed that this sex difference was due to the fact that asthma, apart from acute cases, as a rule only causes slight disability and that moderate degrees of disablement

## Circulatory function

A relatively large number of reports have been published on the effect of chronic obstructive pulmonary disease on circulatory function. As a rule these have concerned studies on series of cases with relatively advanced pulmonary emphysema. Patients with chronic bronchitis or bronchial asthma have usually been represented in the various investigations but in most studies patients with the diagnosis of pulmonary emphysema predominate. It is often clear to the reader however that in many of these patients the primary disease was chronic bronchitis, bronchial asthma or a clinical condition characterized by symptoms typical of both these diseases. No systematic investigations appear to have been made with the aim of following up patients who during a given period were admitted to hospital on one or more occasions for bronchial asthma—with no chronic bronchitis. Neither do any studies appear to have been made for the purpose of correlating several different case-history data concerning the estimated degree of severity of the disease, with the findings made in hemodynamic studies.

Wade and Bishop (1962) compared the results from a number of investigations on the resting cardiac output in chronic bronchitis and pulmonary emphysema. It was pointed out that the designation emphysema referred to patients who had exhibited widely varied clinical pictures with correspondingly varied patterns of disturbed cardio-respiratory function. The comparisons were based on 216 observations in 201 patients with emphysema. In 58 instances the pulmonary arterial pressure was normal, the mean pressure not exceeding 22 mm Hg and in these patients

the mean cardiac index was 3.37 l/min m<sup>2</sup> — In a further 101 observations pulmonary hypertension was present but the patient was not in congestive failure. The mean cardiac index in these patients was 3.43 l/min m<sup>2</sup> — Finally there were 57 observations on patients with pulmonary hypertension who were in congestive failure. In these patients the mean cardiac index was 3.50 l/min m<sup>2</sup> — The presence or absence of congestive failure was not always clearly stated but it was regarded as present if the mean right atrial pressure was greater than 7 mm Hg — The three groups of patients did not differ significantly in the level of their cardiac output which was in each instance essentially the same as that in normal subjects.

Some authors may be mentioned here who have previously studied hemodynamic function in pulmonary emphysema: Borden, Wilson, Ebert and Wells (1950); Mounsey, Ritzmann, Selverstone, Briscoe and McLemor (1952); Harvey, Ferrer, Richards and Courmand (1951); Fowler, Westcott, Scott and Hess (1952); La Lorejoy, Joos, Ny and McCann (1953); Whitaker (1954); and Seherer (1961).

It is evident in several of these investigations that many patients with pulmonary diseases belonging to the chronic obstructive group exhibit residual impairment to circulatory function, that is not rarely incapacitating. As in the case of the lung volume and ventilation capacity studies, however, the conclusions drawn from these reports give no definite guidance with regard to the present problems.

## Socio-medical aspects of chronic obstructive lung diseases

Clausen (1948) in an article entitled "Asthma in Norway" aimed at studying the incidence of the disease and at trying to find its social significance as an incapacitating

increasing age. 20 % of the population between 50 and 60 years of age were deemed disabled. The women were disabled to approximately the same extent as the men, but their disablement appeared to begin at somewhat younger age. 17 % of the population studied by Bentzen were considered to be the asthma-bronchitis-emphysema syndrome. But of these only 28.7 % were assigned to the asthma group, and of this percentage number (the percentage was not stated) had bronchitis, the duration of which had exceeded 3 months.

It would be of great advantage if Bentzen, in discussing the disablement aspect of the material, had attempted differentiation between asthma, chronic bronchitis and emphysema. With the limitations presented in the material this appears to be been possible. No conclusions can be drawn with regard to the group of bronchial asthma patients, from the result given, since this group apparently constitutes less than 1/4 of the material.

Wark (1957) compared the morbidity and mortality in respiratory diseases in England & Wales and Norway. The author reviewed the studies in which epidemiological aspects of respiratory diseases were treated. He also investigated, on the basis of information obtained from several countries, the correlation between the mortality and number of factors such as air pollution, population density, degree of urbanization and industrialization, occupation and different modes of the standard of living. No significant relationship was found, however. The results of the rest of the morbidity data from England & Wales and Norway indicated that there was some difference in morbidity between these countries with regard to bronchitis and pneumonia, but the difference was smaller than that found for the mortality. The author regarded this as an indication that the course of the non-specific respiratory diseases in England and Wales was more serious and that the mortality was higher.

This author studied male transport workers of ages 40-50 years, in Bergen (Norway) and also in London, and compared the prevalence of respiratory symptoms, the ventila-

tion capacity and smoking habits. The London material showed higher prevalence of respiratory symptoms, and at the same time the ventilation capacity was lower. It was not considered that the difference shown between the two series could be explained by difference in anthropometric data or in socio-economic factors. The disabilities in tobacco consumption were assumed to be of significance in the differences in prevalence of a minor respiratory syndrome characterized by cough and the production of phlegm. The difference in the incidences of more serious symptoms ("fatal chronic cardio-respiratory disease") and in the reduction of ventilation capacity was assumed to be due to the difference in exposure to atmospheric pollution between the two series.

Wallup and Connolly (1968) report the incidence of chronic respiratory diseases in the U.S.A., according to the National Health Survey of 1960. Persons with asthma-bronchitis were stated to be approximately six times as common as those with chronic bronchitis but asthma was the reason for only about half many days of bed disability per year per person (5.8 and 5.3 days, respectively). The authors report further the percentage distribution of deaths from chronic respiratory diseases in 1959. Asthma constituted 0.3 % of all causes of death. The corresponding figures for bronchitis and emphysema were 0.2 % and 0.5 % respectively.

The information given can be said to illustrate the relatively great significance attached to asthma in the U.S.A. as compared with chronic bronchitis. To certain extent the conditions in England appear to be the opposite which has been illustrated by several authors, including Wark (1957). Even if there is a true difference between the two countries, it is probable that the data given will be highly influenced by dissimilarities in the diagnostic criteria for these conditions.

For discussion of bronchial asthma and other chronic obstructive pulmonary diseases with regard to occupational aspects, reference may be made to the reviews published by Hauptvedt (1967) and Kowal (1968).

in the men need not result in unemployment, if work is available. For women on the other hand, the author considered it possible that a handicap such as this could have severe consequences.

In an article entitled *Socio-medical aspects of bronchial asthma in Israel* Groen and Lieber (1960) gave some statistical information from hospitals. In a so-called "one day survey" comprising the patients of all hospitals in Israel (with the exception of tuberculosis and mental hospitals) it was found that the bronchial asthma patients constituted 2.2% of those with chronic diseases. When the patients with acute and chronic bronchitis, bronchiectases and cor pulmonale (called "related disorders") were included, the figure rose to 3.6%. The number of hospital admissions for bronchial asthma during the period 1949-1955 constituted 0.5-0.8% of the total if the "related disorders" were included this figure rose to 1.5%. The mean period of hospitalization during the period 1953-1955 for all diseases combined, was 21 days for men and 18 for women. For bronchial asthma the corresponding period was 18 and 11 days, respectively. The number of hospital admissions for bronchial asthma was greater than for malignant gastric tumours and approximately the same as for diabetes mellitus.

Because of the nature of the investigation—the information was obtained from case records and archives of the Israeli hospitals—it was not possible to define the bronchial asthma precisely and to distinguish it from chronic bronchitis, which was the main diagnosis for about half of the patients who were assigned to the group of "related disorders". In the main, however, the information provided by this study appears to contribute much to the elucidation of the problems concerning bronchial asthma and the so-called related disorders.

Hollman (1961) in his article *Das Bronchialasthma als soziale Krankheit* reviewed some reports made by different authors both in West Germany and England on the number of working days lost owing to chronic diseases of the respiratory tract. The number of members of the West German Health Insurance Service who were unfit for work

because of asthma was stated to be 17 for the year 1937, 27 for 1952 and 33 for 1956, per 10 000 insured. The number of days for which sickness benefit was received because of asthma was 320 000 for 1957 for the 2.5 million members.

This information was obtained from the official statistics of the Health Insurance Service, but from a scientific point of view it must be regarded with reservation since the definition and diagnosis of these respiratory diseases probably varied considerably among the doctors making the reports upon which the analysis was based. No attempt was made in this article to define the different conditions. Hollman assumed that the bronchoasthmatic diseases, mainly because of their morbidity would be assigned in the near future to the group of the most common diseases.

Susan Meadows (1961) studied social class migration and chronic bronchitis in male hospital patients in the London area. The author chose a hospital material for her study on the assumption that the bronchitis in these patients would be of a relatively even type. It was found that during the five years immediately preceding the investigation there had been an increase in the number of bronchitic patients in the lower social classes. No corresponding increase was found in a control material. The author believed that the changes of occupation necessitated by the increasing disablement caused by the bronchitis explained the increased number of bronchitic patients in the lower social classes. One possible weak point in this investigation—if attempt is to be made to draw epidemiological conclusions—is that hospital admission as such for this particular disease may have some relationship to the social situation.

Bentsen (1962) studied the sequelae of the chronic obstructive pulmonary diseases in a Norwegian rural population. A person with chronic obstructive pulmonary disease was classified as disabled when the dyspnoea was persistent and so great that he was oppressed by walking at an ordinary pace. The author pointed out that after the age of 50 years the disabling effect of chronic obstructive pulmonary disease increased with

great sex difference was found in this respect.

Ramgren (1932) made a study of the medico-social aspect of haemophilia in Sweden—in 233 persons altogether. The general education of these patients was investigated, and also the types of work by which they were able to earn their living. It was found that of these haemophilia patients only 88 had received an adequate education. One-third had been taught in special schools for the handicapped or had received instruction at home. Practically all of those whose work was of the intellectual or similar type were able to support themselves financially. A large number of those

who had work of the light manual type however were unable to earn their living. 16 / or 33 patients in the entire series were receiving an invalid pension.

The marriage frequency and fertility in patients with mental diseases have been studied by several authors. Only three will be mentioned here namely Essen Möller (1933), Dahlberg (1943) and E. Johansson (1934) all of whom found low marriage frequency and fertility rate. H. Johansson also studied the criminality in a series of male schizophrenic patients and found that there was no significant deviation from the expected figure in normal population.

## *Socio medical aspects of other chronic diseases*

Only a few large systematic investigations of the social aspects of other chronic diseases appear to have been made in Sweden. Reference will be made here to a few investigations of such diseases in which at least some of the indicators of social situation (e.g. marriage frequency, income level, housing standard etc.) which were studied in the present investigation were used.

Smårs and Berfenstam (1961) reported a systematic socio-medical study of 100 individuals with osteogenesis imperfecta. In this disease the incapacity obviously lies in impairment of movement. The general school education had been satisfactory. In studying the different indicators of social situation, information from official statistics was often used for comparisons. The authors found that the marriage frequency in their complete series of cases was lower than for the total population. For the group of patients who were less severely handicapped by the disease it was considered probable that the number of children was only slightly below that of the total population. With regard to income the economically active did not on an average differ to any noteworthy extent from the total population. At the time of the investigation 22 patients (22%) were receiving an invalid pension on account of osteogenesis imperfecta. The housing standard was good. The authors summarized by pointing out the surprisingly good social adaptation often shown by patients with a severe handicap. They considered that the functional impairment was compensated to a great extent by good mental balance and participation in the situation and high intellectual capacity.

Bruce Dahlström and Uggla (1960) made socio-medical studies on patients from Stockholm with another chronic illness, i.e. tuberculosis. The material comprised the 330 hospital inpatients suffering from this disease.

The marriage and divorce frequencies and housing conditions were among the indicators of social situation studied in this investigation. The results were coloured, however, by the large alcohol consumption among a remarkably large number of the patients. Because of this the material as a whole gave the impression of a poorer social situation as judged by the majority of the indicators studied.

Alcoholism also affected the social condition found by Pegelow (1960) in his investigation on 339 consecutive patients receiving hospital treatment for active pulmonary tuberculosis. 27% of the 346 men were classified as chronic alcoholics. This could have explained, for example, the unsatisfactory housing conditions found in a large number of cases, and also the fact that public assistance relief was being received by 65 (26%) of the males at the time of admission to hospital.

Sonja Ritslager Calais (1960) studied the rehabilitation of poliomyelitic patients four years after the onset of the disease in 1953.

Studies were made of the length of the period in hospital, the return to work and occupation at the time of the investigation, changes of occupation by reason of the disease, the re-training frequency, the number of unemployed, economic changes, housing conditions and the frequency of invalid pensioners.

In spite of disease to change their work and undergo re-training on account of their paralysis, several had returned to their old occupations. The social condition appeared to be better among those who, in spite of their handicap had found it possible to return to their former work, probably due to the fact that re-training often involves a change over to more poorly paid work.

Changes of occupation on account of poliomyelitis appeared to be more frequent in the severely disabled categories. On assessing the relationship between general education and return to work it was found that those who were forced to change their occupation had on an average lower education. At the time of the investigation 17 were receiving an invalid pension (ignoring

should have been at least 16 years of age on admission to hospital.

The number of patients who had been admitted to hospital for bronchial asthma and fulfilled the basic criteria was 176 (71 men and 105 women). Three patients were sought in the Loppa County population register where it was found that 13 of them (8 men and 5 women) had died before the 1st November 1960. A further 21 (8 men and 13 women) had moved to another district before this date. The remaining 140 patients were contacted by post or telephone. One man was in hospital for mental diseases, for long-term treatment, and could not therefore take part in the investigation. 4 patients (2 men and 2 women) refused to participate for other reasons. 3 patients (2 men and 1 woman) died and 4 (2 of either sex) moved to another district after the 1st November 1960 before the investigation was begun.

128 patients (48 men and 80 women) thus remained. These patients then underwent examination, which mainly took place between September 1961 and April 1962. During this period 11 of the patients were examined in the out-patients department, and all with the exception of 7 women were admitted to wards for further investigation. Of the 128 patients 13 were diagnosed as having chronic diseases—apart from their asthma—which were considered to have had a decisive influence on their general condition. These 13 underwent the same investigations as the others, but were excluded from the general analyses of clinical and physiological data and from all such medical data with the exception of calculations made with regard to mortality and change of domicile. These latter calculations then comprised all of the patients who on the date of hospital fulfilment of the original criteria for inclusion in the material.

With the aid of the population register and the registry office contact was obtained either by letter or telephone with the majority of those who had moved to other places within 5 years in order to obtain information about their asthma. For those

who died, information on the date and cause of death was obtained.

To summarise there were 176 patients who on admission to hospital during the given period fulfilled the original criteria for inclusion in the material. This figure corresponded to approximately 0.4% of those persons in the population of the areas in question, who were born during the period 1893–1952. The number of those who fulfilled the final criteria for inclusion in the material and who were available for investigation was 128. Of these 12 were diagnosed as having or having had chronic diseases—apart from the asthma—which were considered to have had a decisive influence on their general condition. There thus remained 116 patients who constituted the material on which the main part of the investigation was based (henceforth called the main material).

## Evaluation of the composition and completeness of the material

The aim of this investigation was to study and draw conclusions from a group of patients who had fulfilled certain given criteria, such as age limits and domicile, and who during a given period had been admitted to care in hospitals by reason of bronchial asthma. The material was therefore by probably limited to comprise mainly severe and moderately severe cases of the disease. The completeness of the material will be treated below but firstly a few questions regarding the delimitation of the material will be discussed.



## IV MATERIAL

In order to study the functional sequelae in patients with bronchial asthma it was considered appropriate to select the material from patients who during an exacerbation of the disease had attended a hospital where there were adequate possibilities of differential diagnosis.

### Principles used in the collection of the material

The following principles were applied in the collection of the material

- 1 The patient who should have been resident in Uppsala or in one of seven surrounding communes should have been admitted to one of the Uppsala hospitals mentioned below during the years 1945-58 and should have been judged to be in need of hospital treatment because of bronchial asthma (176 patients; hereafter called the total asthma series). This figure corresponds to approximately 0.4% of the population of the investigation area in the same age groups. The patient should still be resident within the investigation area in November 1960 (140 patients remain).

The patient at the time of admission to hospital, should have been on the popu-

lation register in Uppsala or one of the rural communes surrounding it (North and South Hagunda, Bällinge, Björklinge, Rasbo, Vaksala and Vallholma). By the limitation of the areas from which the patients were taken (hereafter called the collection area) it was considered probable that practically all persons in these areas who were in need of hospital treatment for bronchial asthma would have been referred to hospitals situated in Uppsala. These hospitals would then be either the University Hospital (medical, lung, or ear nose and throat clinics) or the medical department of the Samaritan Hospital.

- 2 The reason for admission to hospital should either be a current attack of bronchial asthma—there should be no grounds for suspecting any degree of cardiac asthma—or to carry out specific hyposensitization. If at the acute stage the patient was being treated in the out patients department

Thus no patients were included for whom the actual reason for admission was the treatment of foci of infection which were suspected of causing the asthma. The latter delimitation was made mainly because these patients often had a mild type of asthma that in itself did not necessitate hospital treatment—in several of these patients the cause of the asthma could not be accepted with certainty.

- 3 The patient should have been born during the period 1893-1932 and

have varied during a period as long as 14 years, due to changes of doctors responsible for the admissions, and to variations in the number of beds available.

In no fewer than 100 of the 176 qualified patients the Unit 1 which at least on one occasion they had filled the requirement for admission—and thereby in known 1 the material—was one and the same 1 the Medical Clinic of the University Hospital, Uppsala. Furthermore this Unit had the same Head for 11 of the 14 years, and it may therefore be assumed that the principles for admission to the department were rather unchanged during this period.

During the period in question the frequency of admission for bronchial asthma in this clinic was practically stationary, the increase in the population of the areas served being taken into account. It is therefore probable that any variations with respect to the indication for admission would have concerned only patients with mild symptoms. If the investigation permits a selection it would be the milder cases that would probably be excluded from the material.

The mean annual number of patients who during the first five years of the qualification period fulfilled the criteria, no admission to hospital, for inclusion in the material, was 124. The corresponding figures for the second five year period and for the remaining four years were 128 and 113 respectively. These figures apply to first time admissions during the period in question. If readmissions are included the last 1 figures in particular have to be corrected to somewhat higher level. The population of persons born between 1890 and 1922, the collection area, increased by approximately 3 during the qualification period.

This therefore supports the view that the indications for admission to hospital were relatively unchanged throughout this period.

### *The possibility of medical care being received outside Uppsala*

The possibility that some patients might have sought medical care in hospitals outside Uppsala must exist. It may be regarded as improbable, however, that an asthma patient faced with an acute situation, when hospital treatment is obviously indicated, would leave his own area and seek advice at a hospital some distance away. It is conceivable, on the other hand, that this patient might attend such a hospital during free or relatively free intervals.

Nine patients with asthma of a degree of severity such that the acute attack could be treated in an outpatients department, and not necessitate admission to a ward were included in the total asthma series because they were later admitted to a ward for hyposensitization. There is a risk, but very small, that similar cases were missed from the recordings by reason of the fact that this hyposensitization was carried out in hospitals situated outside the area served by the Uppsala University Hospital or possibly in an outpatients department. It must be regarded as highly improbable however with the present systems of medical service and the difficulties in arranging for medical care in other hospitals with special departments, that more than a few cases would have been missed in this way.



have varied during a period as long as 14 years, due to changes of doctors responsible for the admissions, and to variations in the number of beds available.

1 no fewer than 106 of the 176 qualified patients the clinic which at least on one occasion they had fulfilled the requirement for admission—and thereby inclusion in the material—was one and the same—the Medical Clinic of the University Hospital, Uppsala. Furthermore this clinic had the same head for 13 of the 14 years, and it may therefore be assumed that the principles for admission to this department were relatively unchanged during this period.

During the period in question the frequency of admissions for bronchial asthma in this clinic was practically stationary, the increase in the population of the areas served being taken into account. It is therefore probable that any variations with respect to the indications for admission would have concerned only patients with mild symptoms. If the investigation permit a selection it would be the milder cases that would probably be excluded from the material.

The mean annual number of patients who during the first five years of the qualification period fulfilled the criteria, on admission to hospital, for inclusion in the material, was 124. The corresponding figures for the second five year period, and for the remaining four years were 128 and 113 respectively. These figures apply to first time admission during the period in question. If recidivists are included the last five figures in particular has to be corrected to somewhat higher level. The population of persons born between 1870 and 1922, in the collection areas, increased by approximately 2 during the qualification period.

This therefore supports the view that the indications for admission to hospital were relatively unchanged throughout this period.

*The possibility of medical care being received outside Uppsala*

The possibility that some patients might have sought medical care in hospitals outside Uppsala must exist. It may be regarded as improbable however that an asthma patient faced with an acute situation, when hospital treatment is obviously indicated, would leave his own area and seek advice at a hospital some distance away. It is conceivable, on the other hand, that this patient might attend such a hospital during free or relatively free intervals.

Nine patients with asthma of a degree of severity such that the acute attack could be treated in an outpatients department, and not necessitate admission to a ward, were included in the total asthma series because they were later admitted to a ward for hyposensitization. There is a risk, but very small, that similar cases were missed from the recordings by reason of the fact that this hyposensitization was carried out in hospitals situated outside the area served by the Uppsala University Hospital, or possibly in an outpatients department. It must be regarded as highly improbable however with the present systems of medical service and the difficulties in arranging for medical care in other hospitals with special departments, that more than a few cases would have been missed in this way.

### *Delimitation of the "collection period" to 1945-1958*

It was chosen to collect the material from a limited period of time those patients being "qualified for inclusion who during a certain period fulfilled certain prerequisites for admission to hospital. The possibility of a patient in the collection area being qualified was obviously dependent upon the length of the qualification period. By extending this period to as long as 14 years the size of the material was considered to be acceptable. The intermittent nature of the disease with sometimes completely or partly symptom free periods which in some cases may extend over years motivated a relatively long qualification period so as to increase the chance of a patient being included in the material.

### *Delimitation of the year of birth to 1893-1932*

By limiting the required year of birth to the given period, it was supposed that some overrepresentation of older patients would be produced. It seemed probable—if at the same time it was presupposed that the asthma had had an early onset—and that the disease in these older persons would have had greater possibilities of influencing the functional sequelae. As will be shown later the majority of patients, regardless of their age at the onset of the disease gradually show improvement and the number with an early onset who still qualified for inclusion in the material was therefore limited.

It may be assumed, for example that many of those who were born between 1900 and 1915 and who were admitted to hospital during the 1930's and first half of the 1940's, would not have returned later and would therefore not be included in the material. The correctness of this assumption is supported by the fact that many of the younger patients with an early onset, who received hospital treatment during the latter half of the 1940's, had such insignificant symptoms by the end of the 1950's that admission to hospital was unnecessary.

Fagerberg (1958) also found a relatively small number with an early age at onset in an adult material of patients with bronchial asthma. In his material there was a relationship between age and age at onset, a higher age being combined on an average with a higher age at onset.

Since one of the aims was to study the effect of the asthma on the social adaptation of the patient it was considered desirable to set the lower borderline for the patient's age at a sufficiently high level for the patient to have lived for some years as an independent member of the community outside the parental home. In choosing the upper age limit, consideration was taken of the fact that persons born in 1893 would have reached the age for old age pension in 1960. It was considered that for patients exceeding this age some difficulties would have been encountered with regard to certain investigations, in obtaining satisfactorily comparable material.

### *Variations in the indications for admission to hospital*

It is obviously possible that the indications for admission to hospital could

have varied during a period as long as 14 years, due to changes of doctors responsible for the admissions, and to variations in the number of beds available.

I no fewer than 106 of the 176 "qualified" patients the clinic at which at least on one occasion they had fulfilled the requirements for admission—and thereby inclusion in the material—was one and the same i. e. the Medical Clinic of the University Hospital, Uppsala. Furthermore this clinic had the same Head for 13 of the 14 years, and it may therefore be assumed that the principles for admission to this department were relatively unchanged during this period.

During the period in question the frequency of admissions for bronchial asthma in this clinic was practically stationary the increase in the population of the areas served being taken into account. It is therefore probable that any variations with respect to the indications for admission would have concerned only patients with mild symptoms. If the investigation permit a selection it would be the milder cases that would probably be excluded from the material.

The known annual number of patients who during the first five years of the qualification period fulfilled the criteria, on admission to hospital, for inclusion in the material, is 134. The corresponding figures for the second five-year period and for the remaining four years were 128, 113, 113 and 113 respectively. These figures apply to first-time admissions during the period in question. If re-admissions are included the last four figures particularly have to be corrected to somewhat higher levels. The population of persons born between 1900 and 1932, the collection years, increased by approximately 3% during the qualification period.

This therefore supports the view that the indications for admission to hospital were relatively unchanged throughout this period.

### *The possibility of medical care being received outside Uppsala*

The possibility that some patients might have sought medical care in hospitals outside Uppsala must exist. It may be regarded as improbable, however, that an asthma patient faced with an acute situation, when hospital treatment is obviously indicated, would leave his own area and seek advice at a hospital some distance away. It is conceivable, on the other hand, that this patient might attend such a hospital during free or relatively free intervals.

Nine patients with asthma of a degree of severity such that the acute attack could be treated in an outpatients department, and not necessitate admission to a ward, were included in the total asthma series because they were later admitted to a ward for hypsensitization. There is a risk, but very small, that similar cases were missed from the recordings by reason of the fact that this hypsensitization was carried out in hospitals situated outside the area served by the Uppsala University Hospital, or possibly in an outpatients department. It must be regarded as highly improbable however with the present systems of medical service and the difficulties in arranging for medical care in other hospitals with special departments, that more than a few cases would have been missed in this way.

### *Delimitation of the collection period to 1945-1958*

It was chosen to collect the material from a limited period of time those patients being "qualified for inclusion who during a certain period fulfilled certain prerequisites for admission to hospital. The possibility of a patient in the collection area being qualified was obviously dependent upon the length of the qualification period. By extending this period to as long as 14 years the size of the material was considered to be acceptable. The intermittent nature of the disease with sometimes completely or partly symptom free periods which in some cases may extend over years motivated a relatively long qualification period" so as to increase the chance of a patient being included in the material.

### *Delimitation of the year of birth to 1893-1932*

By limiting the required year of birth to the given period it was supposed that some overrepresentation of older patients would be produced. It seemed probable—if at the same time it was presupposed that the asthma had had an early onset—and that the disease in these older persons would have had greater possibilities of influencing the functional sequelae. As will be shown later the majority of patients regardless of their age at the onset of the disease, gradually show improvement, and the number with an early onset who still qualified for inclusion in the material was therefore limited.

It may be assumed, for example, that many of those who were born between 1900 and 1913 and who were admitted to hospital during the 1930's and first half of the 1940's, would not have returned later and would therefore not be included in the material. The correctness of this assumption is supported by the fact that many of the younger patients with an early onset, who received hospital treatment during the latter half of the 1940's, had such insignificant symptoms by the end of the 1950's that admission to hospital was unnecessary.

Fagerberg (1958) also found a relatively small number with an early age at onset in an adult material of patients with bronchial asthma. In his material there was a relationship between age and age at onset, a higher age being combined on an average with a higher age at onset.

Since one of the aims was to study the effect of the asthma on the social adaptation of the patient it was considered desirable to set the lower borderline for the patient's age at a sufficiently high level for the patient to have lived for some years as an independent member of the community outside the parental home. In choosing the upper age limit, consideration was taken of the fact that persons born in 1893 would have reached the age for old age pension in 1980. It was considered that for patients exceeding this age some difficulties would have been encountered, with regard to certain investigations, in obtaining satisfactorily comparable material.

### *Variations in the indications for admission to hospital*

It is obviously possible that the indications for admission to hospital could

have varied during a period as long as 14 years, due to changes of doctors responsible for the admissions, and to variations in the number of beds available.

In no fewer than 100 of the 176 qualified patients the clinic at which at least on one occasion they had fulfilled the requirements for admission—and thereby inclusion in the material—was on and the same as the Medical Clinic of the University Hospital, Uppsala. Furthermore this clinic had the same fixed for 13 of the 14 years, and it may therefore be assumed that the principles for admission to this department were relatively unchanged during this period.

During the period in question the frequency of admissions for bronchial asthma in this clinic was practically stationary, the increase in the population of the area served being taken into account. It is therefore probable that any variations with respect to the indication for admission would have concerned only patients with mild symptoms. If the investigation permit a selection it would be the milder cases that would probably be excluded from the material.

The raw actual number of patients who during the first five years of the qualification period fulfilled the criteria, on admission to hospital, for inclusion in the material was 134. The corresponding figures for the second five-year period and for the remaining four years were 126 and 113 respectively. These figures apply to first-time admissions during the period in question. If re-admissions are included the last two figures in particular have to be corrected to somewhat higher levels. The population of persons born between 1893 and 1922, in the collection areas, increased by approximately 25% during the qualification period.

This therefore supports the view that the indications for admission to hospital were relatively unchanged throughout this period.

#### *The possibility of medical care being received outside Uppsala*

The possibility that some patients might have sought medical care in hospitals outside Uppsala must exist. It may be regarded as improbable, however, that an asthma patient faced with an acute situation, when hospital treatment is obviously indicated, would leave his own area and seek advice at a hospital some distance away. It is conceivable on the other hand that this patient might attend such a hospital during free or relatively free intervals.

Nine patients with asthma of a degree of severity such that the acute attack could be treated in an outpatients department, and not necessitate admission to a ward, were included in the total asthma series because they were later admitted to a ward for hypsensitization. There is a risk, but very small, that similar cases were missed from the recordings by reason of the fact that this hypsensitization was carried out in hospitals situated outside the area served by the Uppsala University Hospital, or possibly in an outpatients department. It must be regarded as highly improbable however with the present systems of medical service and the difficulties in arranging for medical care in other hospitals with special departments, that more than a few cases would have been missed in this way.



### *The possibility of medical care being received at home*

The possibility of some patients receiving medical care at home even during severe acute attacks is another factor that should be considered. As in the cases just referred to the likelihood of whether or not hospital care is sought may bear a certain relationship to the degree of severity of the asthma and it may therefore be regarded as probable that the larger proportion of the patients with pronounced symptoms became "qualified." There is reason to assume that home nursing under the supervision of a doctor would only occur in special social groups so that if there had been some patients receiving medical care in this way there would have been a certain degree of selection in the material. It is my opinion, however, that no more than a few isolated cases could have been omitted in this way.

### *The correctness of the diagnosis of bronchial asthma*

Since the primary criterion for a patient's inclusion in the material was that he had attended hospital for acute bronchial asthma and that this acute situation was either a direct reason for his admission to a ward (this applied to 167 patients) or an indirect (this applied to 9 patients) there appears to be no reason for questioning the correctness of the diagnosis, since in most cases there is no difficulty in diagnosing this disease on observation and examination during an actual attack.

One of the main difficulties in diagnosing the symptoms at the acute stage is their differentiation from the symptom picture shown by patients with left cardiac incompetence. Other causes of dyspnoea, e.g. intra- or extra-pulmonary neoplasms, may give rise to a type of respiratory obstruction that may possibly lead to problems in differential diagnosis. In these cases, however, it is highly probable that the patients would return to hospital at a later date and the conditions would be diagnosed then. For details in the differential diagnosis reference may be made to Unger (1932).

The following facts support the correctness of the diagnosis of bronchial asthma in this series of patients:

- a) the onset of acute symptoms was as a rule followed directly—or in exceptional cases after a short period—by admission to hospital, where in any doubtful cases differential diagnosis was possible
- b) careful study of the case histories left the author in no doubt that all the patients in this material had undergone periods of symptoms typical of bronchial asthma

### *Discussion on the evaluation of other complicating chronic diseases and the classification of patients with such diseases*

Many of the patients had at some time suffered from a disease other than asthma. This was either transitory or of a chronic type. An attempt was made from case to case to estimate any influence which this intervening disease might have had on the respiratory and circulatory functions and on the social adaptation of the patient. It was also considered important from a socio-medical aspect

to take into account the fact of whether the disease coincided with a period especially sensitive with regard to social adaptation. It was obviously impossible to establish absolute norms, partly because of the subsequent difficulties in analyzing the influence of these diseases, and partly because these supervening diseases were so varied.

The patients concerned here were divided into two groups. One group comprised those in whom the supervening disease was considered to have had a decisive influence—this group was therefore excluded from the general analyses. The other group which was included in the main material comprised patients in whom no such influence appeared probable. In all except two or possibly three patients in the first group however it was the bronchial asthma that had had the greatest influence on the length of the period of disablement and on the life situation in general. Nevertheless all patients belonging to this group were excluded from the analyses concerning clinical and physiological data, and also those concerning socio-medical data with the exception of the calculation on mortality and change of domicile. It was considered justified to include the patients with other chronic diseases in these two latter analyses, since neither those who had moved nor those who had died, in the asthma series, were investigated in

detail for the possible occurrence of other diseases. The classification of the patients into the two groups according to the clinical assessment is given in an appendix (page 160)

#### Patients excluded from the material because they had moved from the collection area

The 26 persons who had moved to another district did not take part in the main investigation, but were on the other hand included in the calculations of mortality and change of residence. These patients were excluded from the socio-medical analyses because otherwise it would have been necessary to obtain special comparative material for practically each one of them, from the place or places in which they had lived after moving from the collection area. It was not considered justifiable to include them in the investigations on respiratory and circulatory function, partly because of the cost involved in carrying out examinations in hospitals outside patients' own county. Since however it will be shown later the frequency of change of residence was comparatively low and there was no reason to assume that the mean severity of the illness of these persons differed notably from that of those who had not moved from the collection area, the results of the main investigations were probably not affected significantly by the exclusion of these patients. In an attempt to confirm the probability that these patients, from a case history point of view did not constitute an extreme group contacted by post or telephone, with the aid of the population register and registry office, we made with the majority of those who were still living in Sweden.

## V METHODS

The description of the method will be divided into three sections. These sections treat

- 1 Methods for assessment of clinical course with respect to the degree of severity of the bronchial asthma.
- 2 Methods for analysis of circulatory and respiratory function.
- 3 Principles of classification in the treatment of socio medical problems

### Assessment of clinical course with respect to the estimated degree of severity of the bronchial asthma

*Personal interviews with the patients* were carried out by the author mainly during the period August 1961—December 1962 and were based on a previously prepared questionnaire comprising questions on both personal data and the history of the patient's illness. On taking the case history special importance was placed on the estimated number of days of incapacity and symptom days since the onset of this disease. By day of incapacity is meant a day when owing to asthma there was a inability to work estimated as at least 75% and by symptom day at least 25%. It is obvious however that this method of assessing the number of days of incapacity and

symptom days only allowed a rough estimate. Poor memory on the part of the patients for dates that were a relatively long time back, could have been considerable. There appeared however to be no better mode of procedure.

If the recordings that are now to be found in the General Sickness Insurance had extended further back than 1955 it would have been possible to obtain certain information regarding the days when sickness benefit was received. Such information, however, would have been of limited value owing to the intermittent course of the disease because of the number of days that must lapse before application is made for sickness benefit this application is often not made when the symptom periods are short. In spite of this some information could however have been gained from the old sickness benefit service and other benefit funds, but as mentioned above this material was not available for the earlier part of the period.

Attempt was made to compare the information received from the patients with that obtained from the General Sickness Insurance which applied to the period 1955—1960. It was found, however that there was a discrepancy between the days of incapacity noted in the Insurance records and those found in this investigation. As previously mentioned short term illnesses were not always found in the records of the General Sickness Insurance.

Furthermore discrepancies obviously occurred between the recorded number of days when sickness benefit was received and the number of days for which the patient stated that he was disabled to an extent of at least 75% on account of asthma. One reason for the difference may be that the period for which sickness benefit is obtained often includes convalescence days.

By dividing the time after the onset of asthma into periods of a maximum of 5 years, it seemed possible at the interviews to form a good idea in all cases of the effect of the disease with regard to days of incapacity and symptom days. It was particularly difficult however to estimate the number of incapacity and symptom days for those persons who had the type of work in which absence owing to sickness does not result in loss of income e.g. pensioners and housewives. In these cases an assessment was made of the number of incapacity and symptom days that the person in question would have had if he or she had had light, mainly sedentary work.

Assessment of the degree of severity of the disease was made by attributing to each patient a number of sickness points which meant that each day of incapacity was equivalent to 1 point, and each symptom day 1/2 point. The term over all degree of severity will be subsequently used to denote the total number of sickness points since the onset of the disease. The mutual evaluation between day of incapacity and symptom day was obviously somewhat arbitrary but this assessment of their gradation was considered reasonable.

The total number of asthmatic attacks was estimated analogously by the study of each period in turn. The average duration of the attack was determined by means of information from the patient to which of the following groups the majority of the attacks could be assigned.

Group 1 attacks lasting for less than 1 hour

Group 2 attacks lasting 1-3 hours.

Group 3 attacks lasting 3-12 hours.

Group 4 attacks lasting 12-24 hours.

A rough estimate of the total number of attack hours was obtained by multiplying the assessed total number of attacks by the mean figure corresponding to the duration group to which the patient was assigned. Thus a patient who was estimated having had 500 attacks, the duration of most of which was 2-12 hours, had  $500 \times 7.5 = 3,750$  attack hours.

The usual general medical examination was performed by the author personally—as in the case of the interview—both in the outpatients department and during the patient's period in a ward in connection with the follow up examination. These examinations are described in more detail below where a summary of the interview and general examination scheme is given.

5 Summary of questions included in the questionnaire

- 1 The patient's name, date of birth, present address, birth date and occupation
- 2 The year of onset of the bronchial asthma
- 3 The general course of the asthma (improvement, deterioration or no change) since it onset.
- 4 Graduation of the total number of days of incapacity and symptom days on account of the asthma.
- 5 Graduation of the total number of the asthmatic attacks and their average duration.
- 6 The number of times admitted to hospital on account of the asthma.

- 7 The occurrence of chronic bronchitis in the case history
8. Information regarding any other diseases reported in the case history
- 9 Information regarding smoking and alcohol consumption.

*Brief description of the general clinical examination* Physical examination including an assessment of the general condition and body build measurement of height and weight recording of any signs of cardiac decompensation, measurement of blood pressure inspection of mouth and throat palpation of thyroid gland superficial lymph glands and abdomen, auscultation and percussion of heart and lungs and examination of arm and leg reflexes. Examination of blood status comprising Hb content, hematocrit sedimentation rate and differential count of white blood cells. Examination of urine comprising qualitative albumin and glucose tests and sedimentation.

The results of some of these clinical examinations are not reported, since they served only for sorting the material. For example investigations intended to test certain functions were postponed if pronounced anaemia was found in any of the patients. Treatment of the anaemia was commenced and the patient returned when this was cured. Tests of physiological functions were performed if in the women the Hb content was not less than 10.5 g /%, and in the men not less than 11.5 g /%. Women with a Hb content of less than 11.5 g /% and men with a Hb content of less than 12.5 g /% are noted on page 161.

The information given in points 7 and 8 of the questionnaire facilitated sorting out those patients with a other chronic disease—in addition to the bronchial asthma—which was judged to have had a decisive influence on the patient's general situation into of health or functional.

In order to demonstrate changes in the estimated degree of severity of the disease, the average number of sickness points per year was studied for

each patient during the period 1957–1961 and this figure compared with the corresponding figures for the first five years after the onset of the disease, and the period 1947–1951 respectively and also with the average figure for the whole period between the onset of the disease and the end of 1956. The course of the disease in those patients who during early and late periods respectively attained either relatively low or high sickness points was studied. This is discussed in more detail in the reports of the results on page 35.

### Analysis of circulatory and respiratory function

In order to avoid functional changes resulting from a recent asthmatic attack the tests of respiratory and circulatory function were made during a free interval. The latter means that during the period of the tests and for at least two days previously the patient was either entirely free from symptoms or in what he regarded as his most symptom free state for the last twelve months. On auscultation of the lungs during normal breathing there should either be no rhonchi heard or the above-mentioned criterion for the most optimal state should apply.

No symptomatic therapy (with the exception of corticosteroids in those patients who had had long term therapy) i.e. daily doses for the last six months) should have been taken during the 24 hours before testing began. After the work and lung function tests were completed the patients received two inhalations of Isoprenaline spray (Medihaler—Hänsel).

**Electrocardiogram.** ECG was recorded at rest in the supine position, after eight minutes of passive standing, during exercise in the sitting position, and after exercise in the supine position. The following leads were used I, II, III, aVR, VL, VF, V<sub>1</sub>, V<sub>2</sub>, V<sub>3</sub>, V<sub>4</sub>, V<sub>5</sub>, and V<sub>6</sub>. During exercise the left different electrode was moved to the fore head. The ECG-apparatus used was Mingograf II (Elema Schöander Ltd Stockholm).

**DVR.** Basal oxygen uptake was determined under standardized conditions during ten-minute periods, with duplicate determinations on each of two separate occasions. The apparatus used were Sphyrograf Krogh and "Sphyrograf II" (Elema Schöander Ltd, Stockholm). This equipment was used by A. Engström and B. Lund (1961).

**Physical work capacity.** The work test (Sjöstrand, 1947 and W. Åstrand, 1948) was performed on electrically braked bicycle ergometer (Höbomgren and Mattsson, 1933). The heart rate and respiratory frequency were counted, and the ECG was recorded continuously during isometric increases of work load. For the women the test was started at a heart rate of 200 bpm/min and for the men at 300 bpm/min. Each work period lasted for 15 minutes and the work was increased in steps of 200 and 300 kpm/min, respectively until heart rate of approximately 170 bpm/min was reached or until there was some abnormal signs or symptoms. The work test was discontinued. The physical work capacity was arbitrarily defined as the absolute work load performed during steady state at each of the heart rates 170, 140 and 120 bpm/min (W<sub>170</sub>, W<sub>140</sub> and W<sub>120</sub>). Steady state is defined either 10 beats/min or less change of heart rate from the second to the sixth minute of work, or as 2 beats/min or less change from the fourth to the sixth minute. If steady state was not reached, the values for W<sub>170</sub> etc. were calculated nevertheless but are specially indicated in the tables and figures. The value of work capacity was obtained by numerical extra or interpolation using the approximately linear relationship between heart rate and work load. Extrapolation was not performed for more than 20 beats/min. The highest work

load actually performed by the patient for at least 8 min is designated W<sub>max</sub>. The highest heart rate actually attained during the highest work load is designated HR<sub>max</sub>.

The majority of the patients were tested on two separate occasions, twice within the course of six months. Both for W<sub>170</sub> and W<sub>120</sub> the mean value is given for the first and second occasion, separately. When no regard was taken of sex the mean value of W<sub>170</sub> was 402 kpm/min at the first test and 426 kpm/min at the second test. The corresponding values for W<sub>120</sub> were 356 kpm/min and 343 kpm/min, respectively. The standard deviations of the differences between the first and second tests, were for W<sub>170</sub> 85 kpm/min and for W<sub>120</sub> 87 kpm/min. The error of the single determination was therefore 85 kpm/min for W<sub>170</sub> and 87 kpm/min for W<sub>120</sub> (corrected for the difference in means). For W<sub>max</sub> the mean difference between the first and second work test was 31 kpm/min, which means that there was a probable systematic error of 15 kpm/min. For W<sub>max</sub> the systematic error was not of significant order of size. It is noteworthy however that the errors did not lie in the same direction.

The predicted versus normal value for rate of work at heart rate 120 (W<sub>120</sub>) and maximum performed work (W<sub>max</sub>) was calculated for the patients in the tilman series from the values and equations given below. These were obtained from analysis of normal data from the City of Lppsala Health Survey (1961) (Irwin and Linder unpublished observations) age 51-65 years, 25 men and 61 women:

Men  
average W<sub>120</sub> = 650 kpm/min (SD = 158 kpm/min SE = 23 kpm/min)  
average W<sub>max</sub> = 1722 kpm/min, where  $x$  is the age of the patient in years  
(SD = 216 kpm/min SE = 33 kpm/min)  
Women

average W<sub>120</sub> = 411 kpm/min (SD = 118 kpm/min SE = 15 kpm/min)  
average W<sub>max</sub> = 1263 kpm/min, where  $x$  is the age of the patient in years  
(SD = 157 kpm/min SE = 20 kpm/min)

**Heart volume.** The heart volume was determined in the prone position, with the tube

above and the film below the patient. For the frontal projection the central ray was angled. This was originally suggested by Kjellberg Rudhe and Sjöstrand (1919) in their modification of the method of Larsson and Kjellberg (1948). These authors displaced the tube cranially to an angle of 30° to the vertical line. In the present investigation a displacement in the caudal direction of 30° to the vertical line was tried. The result of this modification was empirically compared to that of the method of Kjellberg et al. (1919). In 38 of the present cases, chosen at random from the whole main material, the average value for the total heart volume in the prone position measured according to the present modification, was then found to be  $6.0 \pm 1.8\%$  greater than the average value obtained by the method of Kjellberg et al. (1919).

**Right heart catheterization.** The heart catheterization was usually the last in estimation. The patients had been trained previously to such procedures as breathing through a mouth piece into a Douglas bag and cycling on a bicycle ergometer. One hour before the investigation started the patient received a small oral dose of pentavalent (0.10 g if his weight was below 70 kg and 0.15 g if it was above). Right heart catheterization was performed with the usual technique usually from a left medial cubital vein. The patient lay in the supine position during the whole procedure which included a graded work test on the bicycle ergometer. A double-lumen catheter was used. A polyethylene catheter was placed in the brachial artery. After these procedures were completed the patient rested for approximately 15 minutes. Pressure were then recorded in the brachial artery, the right ventricle, the pulmonary artery and the pulmonary wedge position (PCV pressure). Cardiac output was determined according to the direct Fick principle. Expired air was collected starting three minutes after the insertion of the mouth piece and lasting for 10 minutes. Pressure recordings were made and blood samples taken during the fifth to eighth minutes of gas collection. When these procedures were completed the patient rested again for approximately 15 minutes.

Each alternate patient then received a

infusion of acetylcholine into the pulmonary artery with continuous recording of blood pressures. The dose rate was increased stepwise until a value of about 11 mg/min was reached. Constant infusion of this dose was made for 15 minutes. Expired air was collected during the 5th to 15th minute of infusion. The pressures were measured and blood samples taken during the 8th to 12th minute.

Following this procedure the patient rested for approximately 20 minutes before breathing a mixture of about 11 per cent oxygen (mean 11.32%, lowest 11.20%, highest 11.40%) in nitrogen for 18 minutes. Expired air was collected during the 8th to 18th minute. Pressures were recorded and blood samples were taken during the 13th to 17th minute.

After this procedure the patient rested again for approximately 20 minutes and then breathed a mixture of about 50 per cent oxygen (mean 49.37%, lowest 47.25%, highest 52.28%) in nitrogen. The procedure was the same as during anoxic anoxia.

After a further resting period of approximately 20 minutes each alternate patient, i.e. those who were not given the infusion of acetylcholine, breathed a mixture of about 5 per cent carbon dioxide (mean 5.50%, lowest 5.11%, highest 5.88%) in air. After an equilibration period of 5 minutes, pressures were recorded, blood samples taken and expired air collected during a period of 5 minutes (range 4–8). The patient had a new resting period of approximately 20 minutes and then the whole procedure was repeated with a graded work test as a rule for women 200 and 400 kpm/min and for men 300 and 600 kpm/min. Each work load lasted for 6 minutes unless because of abnormal signs or symptoms the work test had to be ended. The heart rate was determined and pressures recorded very secondarily. Blood samples and expired air were collected during the 4th to 6th minute of work load.

**Lung function tests.** The spirometer used (model Spirokhall Ltd, Stockholm) was a slightly modified version (Berglund, Blom, Björk, Grönby, Kjellner, Samkvist and Söderholm 1965) of that described by Bernstein, D'Silva and Mead (1953). The

procedures for lung volume determination and ventilation capacity were essentially as described by Berglund et al. (1963) Birtath, Kjellmer and Sandqvist (1963) and Grimby and Söderholm (1963). Nearly all the patients were tested on two different occasions with an interval of at least 4-6 months. The determination of the vital capacity (VC) forced expiratory volume in one second ( $FEV_{1.0}$ ) and maximum voluntary ventilation with fixed respiratory frequency of 40 per minute ( $MVV_{40}$ ) or with a respiratory frequency chosen by the patient ( $MVV_p$ ), nearly every patient performed each test twice on the first occasion and three times on the second occasion. By  $FEV_{1.0}$  is meant  $FEV_{1.0}$  expressed as percentage of VC or FVC (forced vital capacity) whichever was the largest  $FEV_{1.0}$  is estimated at the point of the spirogram occurring 0.10 second after the first expiratory deflection, whether the curve immediately assumed its deepest slope or showed initial part of flow acceleration. Before the investigation began the patient was carefully instructed and was allowed initial trial run.

All volumes are given at body temperature and ambient pressure saturated (BTPS). All spirometers were operated by trained nurses. The international accelerometer of lung volumes and test were used.

Since the aim of the investigation was to determine the maximum function, the mean was not calculated. Each case is represented by the largest line obtained at either the first or the second examination.

Determination of total lung capacity (TLC) and its subdivisions was performed with the helium dilution method using closed spirometer (model Spirohelm, KU Ltd, Stockholm). This investigation, however in contrast to the other spirometer tests, was performed only once on each patient. The error of measurement of functional residual capacity for this method has been given by Holmgren (1934) as 80 ml and by Grimby and Söderholm (1963) as 7.5%.

For the three patients the expected normal loss for ventilation capacity and lung volume were calculated from the regression equations (see Table 1) obtained from our and Swedish material, published by Berglund et al. (1963) Birtath et al. (1963) and Grimby and Söderholm (1963). Their values, however were given at ambient temperature and pressure saturated (ATPS). As the widest given range of room temperature and barometric pressure in these experiments was 18°-33° and 740-780 mm Hg, respectively the volumes at BTPS were obtained by multiplying the volumes at ATPS by factor of 1.08-1.12. This factor 1.10 was chosen.

	Sex	Regression coefficients			Constant	RSD
		Age, yrs	Height, m	Wt, kg		
VC (l)	M	-0.020	+ 4.81	—	- 2.81	0.40
	F	-0.022	+ 4.81	—	- 2.25	0.40
$FEV_{1.0}$ (l)	M	-0.033	+ 3.44	—	- 1.80	0.44
	F	-0.037	+ 2.87	—	- 0.54	0.36
$FEV$	M	-0.373	—	—	+ 91.79	7.19
	F	-0.381	—	—	+ 92.51	5.41
$MVV_{40}$ (l/min)	M	-1.368	—	—	+ 180.8	18.8
	F	-0.818	—	—	+ 113.1	18.1
$MVV_p$ (l/min)	M	-1.42	+ 79.0	—	+ 76	30
	F	-0.77	—	—	+ 138	20
TLC (l)	M	—	+ 6.92	-0.017	- 4.30	0.67
	F	-0.016	+ 6.72	—	- 5.77	0.48
FRC/TLC (%)	M	+0.18	—	-0.12	+ 32.3	6.8
	F	+0.18	—	-0.08	+ 48.3	4.7
RV/TLC (%)	M	+0.33	—	-0.14	+ 33.4	4.3
	F	+0.38	+ 27	—	- 28.0	8.1

Table 1 Regression equations and residual standard deviations (RSD) for each lung volume measured in the control material

Source: Grimby and Söderholm (1963)



In the present investigation, as in those of the last mentioned authors, the careful measurement of recorded volumes was not performed until after the end of a patient's examination. The difference between duplicate or triplicate checks was thus not determined exactly during the examination. An examination was therefore not pursued until two identical maximum values were reached. The effect of this procedure was analysed by calculating the difference between the highest and the second highest value for each test—whether it was registered on the first or the second occasion. This difference amounted on an average to 6% of the maximum value for VC, 8% for FEV<sub>1.0</sub>, 8% for FEV<sub>0.5</sub>, 9% for MV<sub>0.5</sub> and MV<sub>1.0</sub>. In the control material (Berglund et al 1963) the above calculated difference amounted to an average of 1–3%, and was therefore lower.

### Analysis of blood samples

Hemoglobin concentration (Hb conc) mg/100 ml was measured spectrophotometrically as cyanmethemoglobin using Actal test solution (Ortho Pharmaceutical Corporation New Jersey). Triple determinations were regularly made. The error of a single determination, calculated from all triple determinations was 1.01 per cent (at an average Hb conc of 13.1 g per 100 ml blood). In 111 of 794 samples one of the triple determinations differed considerably (i.e. by more than 0.3 g/100 ml) from the other two. In such cases the deviant value was rejected, the result being calculated from the other two values. When the deviant values were excluded the error of the method was 0.84 per cent.

Oxygen saturation (S<sub>O<sub>2</sub></sub>) was determined by spectrophotometry of hemolyzed whole blood (double determinations) as described in detail by Holmgren and Pernow (1950). The error of a single determination was 0.0025 units at an average of 1,280 units (unit = ratio of the absorbances at 475 nm and 500 nm). The error corresponded to an average value of 0.23 per cent saturation, if the average oxygen saturation of the blood samples was about 75 per cent.

Oxygen capacity was calculated from the Hb concentration, using a value of 1.34 ml O<sub>2</sub>/g hemoglobin. Oxygen content was calculated from the determined values of oxygen saturation and oxygen capacity with the addition of physically dissolved oxygen.

The lactate concentration in capillary blood was determined (double determinations) spectrophotometrically (Barker and Summerson, 1941 as modified by Sirén, 1949). The error of a single determination was 6.8 per cent at an average lactate concentration of 6.1 mEq per litre blood.

The plasma volume was determined by the method of dye dilution as described in detail by Wiklander (1956) (single determinations). Evans blue dye (T 1824) was injected intravenously and blood samples for spectrophotometric determination were taken at approximately 10 and 60 minutes after the injection. Corrections for loss of dye to the extravascular space (from time zero) and for the expected difference between body hematocrit and peripheral hematocrit (ratio 0.90) were carried out. The error of a single determination was 3.1 per cent, calculated from the individual differences between the 10-minute and 60-minute values, at an average plasma volume of 2.54 litres. The error of the spectrophotometric analysis of a single blood sample was 0.5 per cent.

The hematocrit was determined in microcapillaries (International Hemacrit Centrifuge International Equipment Company Boston) at 10,000 r.p.m. for 5 minutes. No correction for trapped plasma was made, as with this method it is negligible (Garby and Møller 1961).

### Principles of classification in the treatment of socio-medical problems

Summary of the questionnaire used in the interview concerning the socio-medical adaptation of the patient. Each patient was asked for information concerning

- 1 General education.
- 2 Family formation (marriage, children, number of children and number under 18).

years of age on the 1st November 1900 (divorce)

2. Any change of residence and whether this was because of asthma.
4. Any change of occupation and whether this was because of asthma.
6. Whether the patient had discontinued income yielding work and whether the reason for this was asthma.
8. The patient's housing situation on the 1st November 1900, both regards size and standard.
7. Whether invalid pension was being received and whether this was because of asthma.
8. Whether any public assistance money was being received, and whether this was on account of the asthma.
9. The patient's own opinion of his capacity for work during the last year with regard to the asthma.
10. Smoking and alcohol consumption.

*Change of district.* For those members of the population of Uppsala, who on admission to hospital fulfilled the criteria for inclusion in the material, the number who had moved to other district before the 1st November 1900, the number still living in the collection area at this date and the average number of observed years were determined. The number still living in the Uppsala area during the observation period in question was also determined. The Uppsala population was used as control material. By studying the population schedule it was possible to estimate the number who during certain observation periods were still resident in Uppsala. The proband in both the asthma series and the control material were classified according to sex and age. The method is described in more detail on page 119.

*Verifying information on the number who had died in the total asthma series* was obtained from the registry office and population register. In estimating the mortality risk the town population of Sweden was used as control material, for which information was obtained from the official statistics. The

proband both in the asthma series and the control material were classified according to sex and age. The method is described in more detail on page 158.

*General education, family situation, number of children below the age of 16 years, distribution according to branch of industry and occupational status, income-earning among married women, smoking and alcohol consumption.* A short description of the methods is given together with the corresponding reports of the results (cf also appendix I).

*Housing standard and size and income level in 1855 and 1900 according to the distribution in sickness benefit classes by the General Sickness Insurance.* It was desired to compare the asthma series with the rest of the population in the collection area (hereafter called the Uppsala population). It was known that there were differences in sex and civil state between the asthma series and the Uppsala population. Since these factors were correlated both to the sickness benefit class and to the housing size and standard, each individual was ranked in the respective age, sex and civil state groups in the following way:

As an example the procedure in rank class with regard to the housing size (= number of rooms) for a particular person may be described. We take a married man born in 1818, who had a flat consisting of 2 rooms and kitchen. To determine his rank table from the Uppsala Housing Census for the 1st November 1900 we utilized. This table shows that of the married men aged 40-44 years, living together with their wives 806 (41%) live in flats consisting of 2 rooms and kitchen or less, 648 (31.1%) in flat of 3 rooms and kitchen and 501 (24.8%) in flat of 4 rooms and kitchen or more. Those living in 2 rooms and kitchen, of the exemplified person, age, sex and civil state group, should be placed, in consideration of the housing size between 419 and 789. For the sake of simplicity he may be assigned to the middle of this interval, i.e.  $\frac{419 + 789}{2} = 604$ . This means that 60.4% of the Uppsala population are considered to have inferior and 39.6%

more favourable housing conditions as regards size. By this procedure each person was characterized by a percentage figure. If this figure was lower than 50 it implied that the person had less favourable conditions than the mean person in the same age, sex and civil state group. Married female patients living together with their husbands, were assigned according to the age of their respective husbands.

Analogous calculations were made with regard to the distribution into sickness benefit classes. As in the study of the housing standard and size the urban district of Uppsala was treated separately from the rural districts around Uppsala, included in the investigation. The calculations were made from the official statistics of the Uppsala County General Sickness Insurance.

*Definitions used in accordance with the Population and Housing Census 1960.* According to the definitions given in the housing census for 1960 a room is a space measuring at least 6 m<sup>2</sup> containing a window and not intended for the preparation of food. By household is meant the person or group of persons living in a certain house or flat.

Those defined as living in a house or flat are all the persons who for 1961 were registered on the population schedule as being of that address. A household is defined as overcrowded if the number living in the house or flat exceeds 2 per room (the kitchen not included).

According to the population census an income-earning person is defined as a person who during the so-called population census week, carried out income-earning work during at least half of the normal working time. Also regarded as income-yielding were those persons who had income-yielding work but because of temporary discontinuation were not working during the population census week. This discontinuation was regarded as temporary if its duration was no longer than 4 months. These definitions were used in the present study.

The classification of industrial branches was made in accordance with the population census of 1960 and the norms followed were adjusted according to the indices to the international standard industrial classification of all economic activities (ISIC) published in U.N. Statistical Papers, Series M, No. 4.

## VI DESCRIPTION OF CLINICAL COURSE WITH REGARD TO THE ESTIMATED DEGREE OF SEVERITY OF THE BRONCHIAL ASTHMA

The clinical course of the bronchial asthma in this series of patients, with regard to the assessed degree of severity of the disease will now be described. I consider such a description to be motivated as a background to the results, which will be given later concerning the long term effect of the disease on the respiratory and circulatory functions and the social adaptation of the patient.

It was not the aim of the present investigation to take into account the extent to which different types of therapy had influenced the course of the bronchial asthma in this series. All patients were observed in hospital and treated at least by the prevalent type of symptomatic therapy. The subsequent therapy was very irregular. It will only be mentioned here that corticosteroids were used practically daily for period of at least 24 months in 18 patients and that specific hyposensitization was tried in 44 patients.

Table 2 shows the variation ranges and arithmetic mean values for the age at onset, duration of the disease, number of days of incapacity and symptom days respectively (see page 24), sickness points (see page 25), number of attacks and total number of attack hours, both totally and distributed according to age and sex.

The figures in the table, which applies to the end of 1961 show that the

mean age for both men and women was 34 years. The mean age at onset for the men was 32 years and for the women 33 years, and this increased in direct relationship to age, a higher age group having a higher mean age at onset. This applied to both men and women. The total mean duration up to the end of 1961 was 21 years for both men and women. This duration was practically the same in the different age groups. By total duration is meant that time in years that had elapsed since the onset, minus the number of completely symptom-free calendar years. This involved some uncertainty but for practical reasons this form of assessment was necessary. The mean number of completely symptom free calendar years was negligible.

The mean number of days of incapacity and symptom days was about 1000 and 2000 respectively for both men and women. The mean total number of sickness points was about 1700 for both men and women. There appeared to be no great difference between the different age groups, but in the women in the younger age group however the mean number of points was relatively low.

1 112 of the 116 men and women the

asthma was of the type that involved for the greater part pronounced attacks of symptoms, i.e. episodic symptoms. For these patients the mean number of attacks was about 5.0. In the main there was no sex difference in this respect. Among the different age groups it was noted that in the younger men the mean number of attacks was relatively high but in the older men relatively low. In contrast to the younger

men, the younger women had a relatively low mean number of attacks.

The mean number of attack hours was about 3900 for the men and about 3500 for the women. The highest number was noted for the younger men, while the lowest was found in the group of younger women. In this respect, as for all the studied factors mentioned above the ranges within the sex and age groups were very large.

Case history factor groups acc. to sex and yr of birth					Case history factor groups acc. to sex and yr of birth				
		No	range	mean			No.	range	mean
<i>Age yrs</i>					<i>Symptom days</i>				
Total	M and F	116	29-68	54	Total	M and F	116	76-11460	2090
	M	42	32-68	54		M	42	76-11460	2168
	F	74	29-68	54		F	74	105-7680	2030
M born	1912-1932	13	32-49	39	M born	1912-1932	13	484-3360	1808
" "	1902-1911	18	30-59	55	" "	1902-1911	13	315-11460	2698
" "	1893-1901	16	60-68	64	" "	1893-1901	16	76-5940	2037
F "	1912-1932	29	20-49	42	F "	1912-1932	29	210-3570	1563
" "	1902-1911	17	50-59	53	" "	1902-1911	17	105-4980	2036
" "	1893-1901	28	60-68	65	" "	1893-1901	28	222-7680	2513
<i>Age at onset yrs</i>					<i>Sickness points</i>				
Total	M and F	116	2-64	33	Total	M and F	116	79-7781	1696
	M	42	2-64	32		M	42	79-7781	1681
	F	74	2-60	33		F	74	79-6353	1704
M born	1912-1932	13	2-41	17	M born	1912-1932	13	79-3547	1690
" "	1902-1911	13	4-49	37	" "	1902-1911	13	198-7781	1714
" "	1893-1901	16	4-64	44	" "	1893-1901	16	175-4509	1728
F "	1912-1932	29	2-42	22	F "	1912-1932	29	189-3581	1802
" "	1902-1911	17	26-51	36	" "	1902-1911	17	70-6353	1818
" "	1893-1901	28	12-60	41	" "	1893-1901	28	193-4582	1859
<i>Duration of disease yrs.</i>					<i>No. of attacks</i>				
Total	M and F	116	1-84	21	Total	M and F	116	1-4320	556
" "	M	42	1-50	21	" "	M	40	1-4320	537
" "	F	74	1-54	21	" "	F	73	1-3720	567
M born	1912-1932	13	1-37	21	M born	1912-1932	12	1-4320	607
" "	1902-1911	13	4-50	23	" "	1902-1911	13	1-2860	517
" "	1893-1901	16	4-41	26	" "	1893-1901	15	20-1600	427
F "	1912-1932	29	2-47	11	F "	1912-1932	29	1-1900	424
" "	1902-1911	17	1-32	19	" "	1902-1911	17	1-3720	730
" "	1893-1901	28	5-64	23	" "	1893-1901	27	1-3115	606
<i>Days of incapacity</i>					<i>Attack hours</i>				
Total	M and F	116	0-1010	1008	Total	M and F	113	8-34980	3840
	M	42	0-1000	960	" "	M	40	8-34980	3874
	F	74	0-1010	1035	" "	F	73	8-23254	3520
M born	1912-1932	13	0-2550	973	M born	1912-1932	12	8-34980	5120
" "	1902-1911	13	14-1000	827	" "	1902-1911	13	8-22680	3120
" "	1893-1901	16	180-3300	1057	" "	1893-1901	15	40-11840	3532
F "	1912-1932	29	0-2430	877	F "	1912-1932	29	8-8640	2166
" "	1902-1911	17	45-4910	1147	" "	1902-1911	17	16-18218	4668
" "	1893-1901	28	120-3300	1130	" "	1893-1901	27	16-23534	4252

Tabl. 2. Mean values and ranges for characteristics of the material at the end of 1961 with regard to sex and age and some case history data.

Three patients whose asthma was not of the attack type are excluded from the Table.

Table 3 divides the age and sex groups into groups according to the estimated total number of sickness points. Group A consists of patients who did not attain 1000 points, group B those who attained 1000 but not 2000 and group C those who attained 2000 sickness points or more. For

those belonging to group A the mean number of sickness points was 5.3 for those in group B 14.70 and for those in group C 30.89. Of the patients in group A, two had fewer than 100 points. On the whole there was even distribution between the groups for both men and women.

Y. of birth	MALE				FEMALE			
	A <1000 p	B 1000— 1999 p	C ≥2000 p	A+B+C	A <1000 p	B 1000— 1999 p	C ≥2000 p	A+B+C
1912—1933	2	6	5	13	12	11	6	29
1902—1911	7	2	4	13	8	7(5)	5(4)	17
1892—1901	2	9	5	16	9	7(5)	12(11)	28
T. total	12	16	14	42	29	25	23	74

Table 3 The series distributed according to sickness point (classes A, B and C), year of birth and sex. Sickness point valid at the end of 1961 (in parentheses the corresponding distribution 1957 when the differs from the distribution 1961)

What form has the asthma course taken since its onset? Has the disease tended towards deterioration or improvement or has there been no noteworthy change? When during the present investigation these questions were put to each of the patients, the great majority about 80%, stated that on the whole the disease had shown improvement. Only a small number about 5% considered that their asthma had become worse during the years since its onset. This period, however varied greatly among the patients, and it was therefore found that detailed analysis on the basis of these norms would not satisfactorily illustrate the course of the asthma in this series. It was decided instead to classify the patients both according to their age at the time of the investigation and their age at the onset of the disease and to study for each patient

the average number of annual sickness points for certain periods as given below

Point group comprised those patients who during the period in question attained on an average fewer than 50 sickness points per year group B those who attained 50 but not 100 points and group C those who attained 100 points or more. The following comparisons were made

1. Comparison between the sickness point for the first 5 years after onset and those for the 5-year period almost immediately preceding the time of the present investigation, i.e. the years 1957—1961
2. Comparison between the sickness point for the period 1947—1951 and those for the years 1957—1961.
3. Comparison between the sickness points for the period from the onset of the asthma up to 1957 and those for the period 1957—1961

Patients with an observation period of less than 10 years were excluded from these comparisons.

For the patients who during a groups with the lowest and highest certain period had belonged to the average annual sickness points, re-

Time period and population studied	Year of birth											
	1893—1911					1912—1937						
	Age at onset											
	≤10	17—30		≥40		≤16	17—30					
	Sickness points group											
	a	b	c	a	b	c	a	b	c	a	b	c
Distribution of patients into severity groups during the first 5 years after the onset	1	3	1	5	14	10	7	15	10	7	8	2
Corresponding distribution during the period 1937—61	3	1	1	15	6	8	20	0	3	12	3	2
No. of patients who during the first 5 years after the onset had slight <sup>a</sup> asthma.	1			3			7			7		
Their distribution into groups by degree of severity during the period 1937—61	1			3	3		5	2		6	1	
No. of patients who during the first 5 years after the onset had severe <sup>c</sup> asthma.			1		10				10		2	
Their distribution into groups by degree of severity during the period 1937—61			1		3	3	4		2	1		
No. of patients who during the period 1937—61 had slight <sup>a</sup> asthma.	3			15			20			12		
Their distribution into groups by degree of severity during the first 5 years after the onset.	1	2		3	0	3	5	8	7	6	0	
No. of patients who during the period 1937—61 had severe <sup>c</sup> asthma.			1		8		3			3		1
Their distribution into groups by degree of severity during the first 5 years after the onset.			1		4	4		2	1		2	1

Table 4. Comparison between the distribution of the patients at two different periods. Grouping according to the degree of severity of the asthma.

The Table shows classified by year of birth and age at onset of the asthma, the number of patients in three severity groups ("slight asthma"—a, moderately severe—b, and severe—c) according to sickness point comparison being made between the first 5 years after the onset of the asthma and the period 1937—61. The Table also shows the distribution of the severity groups during 1937—61 of those who during the first 5 years after the onset belonged to the groups with the lowest and highest mean annual sickness points, respectively. Also shown in the Table is the distribution of severity groups, during the first 5 years after the onset of those who during 1937—61 belonged to the groups with the lowest and highest mean annual sickness points, respectively.

spectively the distribution between the different severity groups during an earlier or later period of time was studied. It was thus possible not only to study the distribution according to severity within the group but also the development in the group from one period to another.

As shown in Tables 4 and 5 there was an over-all impression of improvement regardless of whether the observation times comprised the first

5 years after the onset and the period 1957-1961 or the two periods 1947-1951 and 1957-1961. Division into groups according to age and age at onset showed that this tendency was common to all groups. The same impression was also obtained on comparison of the sickness point groups for the whole period between the onset up to the end of 1956 with the corresponding groups for the period 1957-1961 (see Table 6).

Time period and population studied	Year of birth				
	1903-1911			1912-1921	
	Age at onset				
	≤16	17-30	≥40	≤16	17-30
	Sickness points group				
	b	b	b	b	b
Distribution of patients into severity groups during the period 1917-51	1 3 1	5 10 11	4 8 12	4 8 3	2 8 4
Corresponding distribution during the period 1957-61	3 1 1	15 6 8	15 7 2	12 3 2	10 3 1
No. of patients who during the period 1917-1951 had "slight" illness	1	8	4	4	2
Their distribution into groups by degree of severity during the period 1957-61	1	4 1	3 1	4	2
No. of patients who during the period 1917-51 had "severe" asthma	1	11	12	5	4
Their distribution into groups by degree of severity during the period 1957-61	1	5 3 6	2 2 2	2 1 2	2

Table 5 Comparison between distributions of the patient at two different periods. Grouping according to the degree of severity of the asthma.

Classifications. In Table 4, Comparison is made between the period 1917-51 and 1957-61. The Table also shows the distribution into severity groups during 1917-51 of those who during the period 1917-51 belonged to the groups with the lowest and highest mean annual sickness points, respectively.



Comparison between Tables 4 and 5 appears to indicate that the situation in 1917-1931 was somewhat worse than during the first five years after the onset. This may possibly be explained by the selection principles—those who were admitted to hospital

in a period which constituted part of the qualification time belonged relatively often to a group with a high mean number of sickness points for this period.

On studying the development in those patients (20 altogether) who during the first

Time period and population studied	Year of birth														
	1893—1911						1912—1932								
	Age at onset														
	≤16			17—39			≥40			≤16			17—39		
	Sickness points group														
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Distribution of patients in severity groups during the total duration up to and including 1936.	3	1	1	4	15	10	6	15	11	4	11	2	4	9	7
Corresponding distribution during the period 1937—61	3	1	1	15	6	8	20	9	3	12	3	2	15	1	1
No. of patients who during the total duration up to and including 1936 had slight asthma.	3			4			6			4			4		
Their distribution into groups by degree of severity during the period 1937—61	3			3	1		6	1		4			2	2	
No. of patients who during the total duration up to and including 1936 had severe asthma.			1		10			11			2			7	
Their distribution into groups by degree of severity during the period 1937—61	1			3	2	5	6	3	2	1	1		5	1	1

Table 6. Comparison between distribution of the patients at two different periods Grouping according to the degree of severity of the asthma.

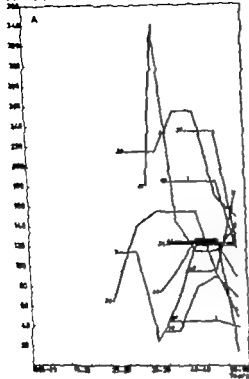
Classifications as in Table 4. Comparison is made between the period which had elapsed from the onset of the asthma up to 1937 and the period 1937-61. The Table also shows the distribution into severity groups, during 1937-61 of those who during the period from the onset of the asthma up to 1937 belonged to the groups with the lowest and highest mean annual sickness points respectively.

Fig. 1. Changes in the estimated degree of severity with regard to the mean annual number of sickness points for 5-years periods since the onset of the asthma for patients in certain selected groups.

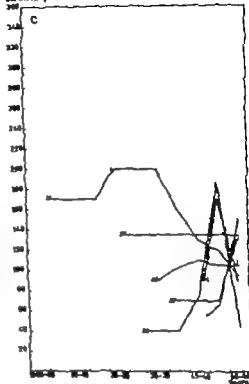
The figure at the start of curve line denotes age at onset. The arrows indicate the years during which invalid pension was received.

Fig. 1 A shows the course for invalid pensioned women, Fig. 1 B for women belonging to the group with  $\geq 2000$  sickness points (excluding the invalid pensioned). Fig. 1 C for invalid pensioned men, and Fig. 1 D for men belonging to the group with  $\geq 2000$  sickness points (excluding the invalid pensioned).

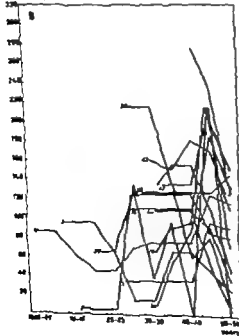
S. phoenicea points



S. phoenicea points



S. phoenicea points



S. phoenicea points

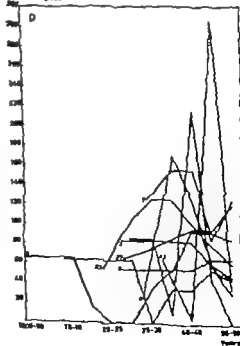


Fig 1

Comparison between Tables 4 and 5 appears to indicate that the situation in 1917-1931 was somewhat worse than during the first five years after the onset. This may possibly be explained by the selection principles—those who were admitted to hospital

in a period which constituted part of the qualification time belonged relatively often to a group with a high mean number of sickness points for this period.

On studying the development in those patients (30 altogether) who during the first

Time period and population studied	Year of birth														
	1893—1911						1912—1932								
	Age at onset														
	≤16			17—39			≥40			≤16			17—39		
	Sickness points group														
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Distribution of patients into severity groups during the total duration up to and including 1936.															
Corresponding distribution during the period 1937—61	3	1	1	4	15	10	0	15	11	4	11	2	4	9	7
	3	1	1	15	0	8	20	0	3	12	3	2	15	4	1
No. of patients who during the total duration up to and including 1936 had slight asthma.	3			4			0			4			4		
Their distribution into groups by degree of severity during the period 1937—61	3			3	1		5	1		4			2	2	
No. of patients who during the total duration up to and including 1936 had severe asthma.			1		10			11			2				7
Their distribution into groups by degree of severity during the period 1937—61		1		3	2	5	6	3	2		1	1	5	1	1

Table 6. Comparison between distribution of the patients at two different periods. Grouping according to the degree of severity of the asthma.

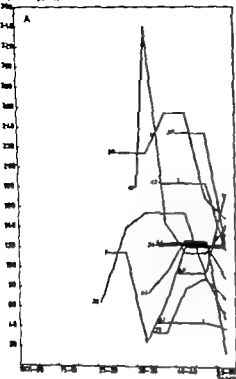
Classifications as in Table 4. Comparison is made between the period which had elapsed from the onset of the asthma up to 1937 and the period 1937-61. The Table also shows the distribution into severity groups, during 1937-61 of those who during the period from the onset of the asthma up to 1937 belonged to the groups with the lowest and highest mean annual sickness points, respectively.

Fig. 1. Change in the estimated degree of severity with regard to the mean annual number of sickness points for 5-years periods since the onset of the asthma for patients in certain elected groups.

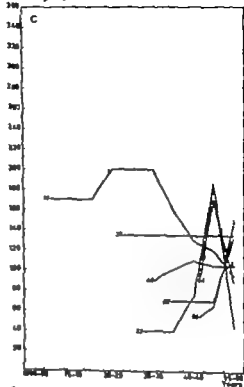
The figure at the start of curve line denotes age at onset. The arrows indicate the years during which invalid pension was received.

Fig. 1 A shows the course for invalid pensioned women, Fig. 1 B for women belonging to the group with  $\geq 2000$  sickness points (excluding the invalid pensioned). Fig. 1 C for invalid pensioned men and Fig. 1 D for men belonging to the group with  $\geq 2000$  sickness points (excluding the invalid pensioned).

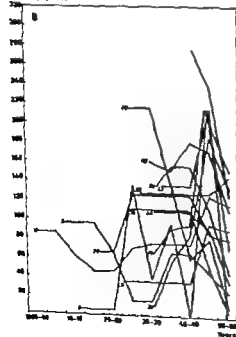
Sclerostoma parvum



Sclerostoma parvum



Sclerostoma parvum



Sclerostoma parvum

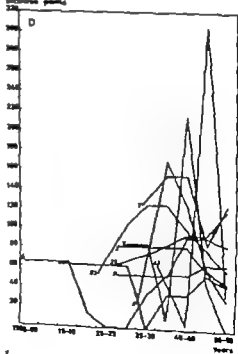


Fig 1

five years after the onset belonged to group a, it was found that only one was assigned to group a during the period 1937-1961 i.e. only one showed marked deterioration (see Table 4). A corresponding study of those who belonged to group c from the beginning (30 patients) showed that 16 of these were to be found in group a in the period 1937-1961 while 7 remained in group c. Table 4 suggests that the development in the patient with an onset on or before the age of 16 years does not appear to be so favourable as that in the patients with a later onset age.

Table 4 show further that of those in whom the onset occurred on or before the age of 10 years and who in 1937-1961 belonged to group a not one belonged to group c during the first five years after the onset and that none of those who were in group c in 1937-1961 belonged to group a during the first 5 years after the onset of the disease. Of the 13 patients—regardless of age or age at the onset of the asthma—who belonged to group a in 1937-1961 there was only one in group a during the first 5 years after the onset.

Thus for the patients in this material there appeared to be some relationship between the degree of severity of the asthma during the first 5 years after the onset and that during the period 1937-1961. This relationship appeared to be more striking in those in whom the asthma was first manifested at or before the age of 16 years.

The picture of the course of the bronchial asthma with regard to its assessed degree of severity shown in Tables 4-6 is complemented by Fig. 1. This shows the course from the time of onset up to the time of the present investigation for each of the patients who received an invalid pension solely or mainly by reason of bronchial asthma and also for those who according to the group classification in

Table 3 belonged to group C, i.e. those who during the period since the onset of the asthma had attained 2000 sickness points or more.

As the figure shows, the trend varied for different patients and the intensity of the disease in one and the same patient often varied greatly in different periods. It is also shown that a favourable development with regard to the intensity of the disease in the majority of patients is characteristic of the years immediately preceding the time of the present investigation.

In this description no consideration was taken of the extent to which different forms of therapy had influenced the course. It seemed obvious however that the favourable development with respect to the patient's symptoms was greatly due to the therapeutic advances made in recent years. But it is also clear that in many patients the disease showed a spontaneous tendency towards improvement. The tables and figures show that it was essentially during the years preceding the time of this investigation that the improvement took place.

### Relationship between pairs of case history factors

In order to illustrate the relationship between different case history factors, a regression analysis was performed. This comprised all of the eight factors that are given in the quantification description below. Table 7 shows the relationship between pairs of case history factors expressed both by corre-

lation coefficients and by significance asterisks.

Between, for example, the variable for the total number of sickness points and that for the number of admissions to hospital, there was positive correlation coefficient of 0.21. This meant that for the patients with high total number of sickness points and

admission to hospital was more common than for patients with low number.

Between the variable for the total number of sickness points and that for the age at onset there was negative correlation of -0.22. This meant that the mean age at onset was lower for patients with high total number of sickness points than for patients with low number.

The different case history factors are quantified as below:

$X_1$  is the sex variable

$X_1 = 0$  for women (74 patients)

$X_1 = 1$  for men (42)

$X_2$  is the age variable

$X_2 = 1$  for patients 60-69 years old (41 patients)

$X_2 = 2$  50-59 (30)

$X_2 = 3$  30-49 (42)

$X_3$  is the variable for the total number of sickness points

$X_3 = 1$  for < 1000 points (38 patients)

$X_3 = 2$  1000-1999 (41)

$X_3 = 3$   $\geq$  2000 (37)

$X_4$  is the variable for duration

$X_4 = 1$  for < 15 years (32 patients)

$X_4 = 2$  15-24 (41)

$X_4 = 3$   $\geq$  25 (37)

$X_5$  is the variable for age at onset

$X_5 = 1$  for  $\leq$  16 years (22 patients)

$X_5 = 2$  17-30 (31)

$X_5 = 3$   $\geq$  31 (42)

$X_6$  is the variable for the number of attack hours

$X_6 = 1$  for 1-499 hours (37 patients)

$X_6 = 2$  500-2499 (20)

$X_6 = 3$   $\geq$  2500 (44)

$X_7$  is the variable for the mean number of sickness points per year during the period 1947-1981

$X_7 = 1$  for < 50 points (10 patients)

$X_7 = 2$  50-99 (27)

$X_7 = 3$   $\geq$  100 (18)

$X_8$  is the variable for the number of times admitted to hospital

$X_8 = 1$  for 1-4 admissions (31 patients)

$X_8 = 2$  5-9 (21)

$X_8 = 3$   $\geq$  10 (14)

Case history factor	$X_1$	$X_2$	$X_3$	$X_4$	$X_5$	$X_6$	$X_7$	$X_8$
Sex	$X_1$	-0.05	0.05	0.02	-0.07	0.01	0.06	0.08
Age	$X_2$	-0.05	-0.12	-0.08	-0.58**	0.04	-0.10	-0.15
Total sickness points	$X_3$	0.05	-0.12	0.59***	-0.32***	0.60***	0.32***	0.31***
Duration	$X_4$	0.02	-0.08	0.59***	-0.51***	0.37**	0.13	0.17
Age at onset	$X_5$	-0.07	-0.58***	-0.32***	-0.51**	-0.27**	-0.01	-0.04
Attack hours	$X_6$	0.01	0.04	0.60***	0.27**	-0.27*	0.30	0.35***
Sickness points 1957-61	$X_7$	0.06	-0.10	0.32*	0.13	-0.01	0.20	0.31**
Admissions to hospital	$X_8$	0.08	-0.15	0.31***	0.17	-0.04	0.35***	0.34***

Table 7 Correlation coefficients — with significance asterisks — between pairs of case history factors

$X_1$ — $X_8$  refer to the different case history factors.

Significance test as in point 7 page 159

The table shows that there is a highly significant relationship between different pairs of case history factors. As might be expected, it is found that the total number of sickness points is the factor that, paired with others, shows the highest number of significant relationships.

The sex factor shows only insignificant relationships with pairs of other factors.

There is a significant relationship between the age factor and the age at onset due to the fact that in this series the patients with a relatively high mean age also had a relatively high age at onset.

The total number of sickness points is highly significantly correlated to the duration, age at onset, attack hours and sickness points during the five-year period almost immediately preceding the time of the investigation.

1. e. 1957-61 and to the number of hospital admissions on account of bronchial asthma. This means that on an average the patients with a high total number of sickness points also had a long duration, a low age at onset, a high number of attack hours, a high annual number of sickness points during the years 1957-61 and a high number of admissions to hospital.

The duration is highly significantly correlated to the age at onset, and significantly correlated to the number of attack hours. This means that patients with a long mean duration had an early age at onset and a high number of attack hours.

The age at onset is significantly correlated to the number of attack hours, patients with a high age at onset having on an average a low number of attack hours.

The number of *attack hours* is highly significantly correlated to the number of admissions to hospital, patients with a high number of attack hours having on an average a high number of admissions to hospital.

The number of *sickness points* during the period 1957—61 is highly significantly correlated to the number of admissions to hospital, patients with a high number of sickness points during this period having on an average a high number of admissions to hos-

pital during the whole period since the onset of asthma

---

Since the total number of sickness points shows a highly significant correlation to all the other factors that are characteristic of the case history of an asthma patient, the grouping of the patients in the main part of the subsequent analysis of the results will be based on the total number of sickness points.



## VII RESPIRATORY AND CIRCULATORY FUNCTION IN RELATION TO THE CLINICAL COURSE AND ESTIMATED DEGREE OF SEVERITY OF THE BRONCHIAL ASTHMA

The results of the investigations on the effects of bronchial asthma on respiratory and circulatory function in the patients in this series are in principle divided into three sections. Because of the close relationship between the cardiovascular and respiratory functions however no sharp line of demarcation is drawn between them. The first section treats ventilation capacity and lung volumes, the second physical work capacity and some aspects of its relation to lung function and the third section hemodynamics and some aspects of its relation to lung function.

Those patients who had or had had another chronic disease — in addition to the bronchial asthma — which might have had a definite influence on the respiratory and circulatory functions, are included in the general analyses of the results and the discussions. The results obtained from investigations in these patients are reported separately when considered of interest.

Before reporting the respiratory and circulatory data obtained it should be emphasized that in the patients to whom these data apply no abnormal symptoms of cardiac decompensation or coronary insufficiency were present at the time of the present investigation. No definite signs of hypertrophy or decompensation of the left or right ventricle were found either on physical or roentgenologic examination or on ECG. Neither were there any definite signs of alveolar or

coronary heart disease in the patient included in the general analyses. As in all had previously a separate report is made on those patients in whom abnormal conditions such as these were observed this category also includes patients with more pronounced pulmonary changes due e.g. to tuberculosis, seen roentgenologically and also those who had or had had chronic bronchitis. The number of patients with signs and symptoms of a noteworthy type was 12. The case histories of these patients as for those in whom there were signs of an additional disease — not however assessed as being of decisive importance — are given on pages 160-164.

In Table 8 some anthropometric data are given for the patients in the asthma series, grouped according to age and sex. Figs. 2, 3 and 4 show the relationships between different dimensions in the cardiovascular system with the patients grouped according to their estimated total number of sickness points (see page 26). The mean circulatory function capacity (expressed as  $W_{150}$ -load in lpm/min at heart rate 130 beats/min) was found to be lower in patients with a high number of sickness points than in those with a low number. This difference was statistically significant. In none of these groups of patients was the total Hb content (THb) found to vary significantly with the circulatory function capacity. This means thus that the ratio

$W_{120}/Tlhb$  was on an average somewhat lower for patients with a high than with a low number of sickness points, but the difference was not, however statistically significant. Yet

there were the relationships between the total cardiac volume and  $W_{120}$  and  $Tlhb$  respectively significantly influenced by the number of sickness points.

Age	Sex	Height cm	Weight kg	BSA m <sup>2</sup>	W <sub>120</sub> ml	Heart ml	Tlhb g	Tlhb g/kg	W <sub>120</sub> per 100 ml	Blood g	Blood ml/kg
<b>Male</b>											
20-29	No. men	13	13	13	13	13	13	13	13	13	13
	mean	173	72	1.85	+4.8	613	676	9.94	14.79	5.29	73.7
	highest	191	91	2.30	+21	835	982	15.71	16.19	7.53	118.4
	lowest	163	53	1.56	-6	395	411	8.87	12.74	3.07	49.8
30-39	No. men	13	13	13	13	13	13	13	13	13	13
	mean	170	70	1.80	+6.3	610	679	9.32	14.57	4.39	63.5
	highest	185	93	2.13	+26	733	829	11.81	16.41	6.80	87.8
	lowest	151	48	1.53	-11	408	483	5.37	11.70	3.39	42.7
40-49	No. men	16	16	16	16	16	16	16	16	16	16
	mean	173	71	1.83	+9.1	631	633	8.80	14.58	4.77	67.7
	highest	191	88	2.08	+43	883	906	12.50	16.98	6.73	96.8
	lowest	161	56	1.68	-10	515	437	6.25	11.99	3.33	48.6
Total	No. men	42	42	42	42	42	42	42	42	42	42
	mean	172	71	1.84	+8.8	628	633	9.02	14.62	4.82	69.0
	highest	191	91	2.30	+43	833	982	15.71	16.98	7.53	118.4
	lowest	151	48	1.52	-11	395	408	5.37	11.70	3.07	42.7
<b>Female</b>											
20-29	No. women	20	20	20	20	20	20	20	20	20	20
	mean	162	61	1.66	+2.9	503	431	6.50	12.16	3.63	55.1
	highest	189	86	1.91	+22	635	610	9.83	13.89	4.86	79.4
	lowest	133	47	1.41	-23	278	303	4.93	10.61	2.72	33.0
30-39	No. women	17	17	17	16	17	15	16	14	16	15
	mean	160	64	1.66	+0.3	513	463	7.36	12.92	4.01	63.9
	highest	171	80	1.83	+14	699	609	11.10	14.88	6.00	100.0
	lowest	152	50	1.58	-11	309	312	4.05	10.63	3.36	49.2
40-49	No. women	23	23	23	23	27	28	28	28	28	29
	mean	161	66	1.66	+9.5	550	561	7.74	13.66	4.03	62.1
	highest	171	94	2.01	+48	920	826	12.36	15.83	6.50	91.9
	lowest	133	41	1.38	-11	365	239	5.07	11.00	3.90	43.1
Total	No. women	71	71	71	70	72	69	69	68	69	69
	mean	161	63	1.66	+4.9	524	467	7.31	12.92	3.99	61.5
	highest	171	91	2.01	+48	920	826	12.36	15.83	6.50	100.0
	lowest	133	41	1.38	-23	365	239	4.05	10.63	2.72	33.0

Table 8 Mean values, highest and lowest values of some anthropometrical data.

I have used the following tables showing age, in this chapter the ages of the patients refer to 1st Jan 1952. One woman born in 1902, and who had not quite reached the age of 30 years, is combined in this and the following tables showing age distribution with the 30-year old patients.

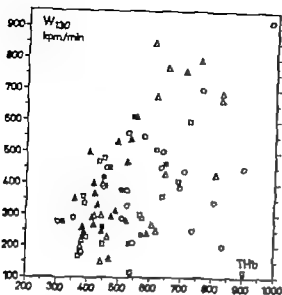


Fig 2 The relationship between rate of work at a heart rate of 130 beats/min ( $W_{130}$ ) kpm/min, and total amount of hemoglobin, g

Triangles represent patients who belong to group A (page 33) squares those belonging to group B circles those belonging to group C. Open symbols represent patients who have been at a steady state at a heart rate of 130 beats/min, and filled symbols those who have not

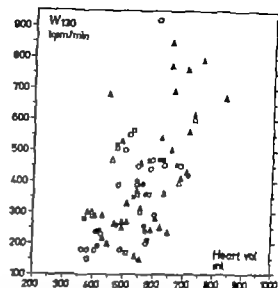
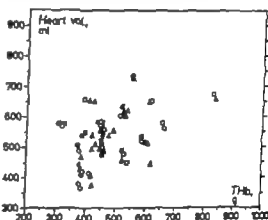


Fig 3 The relationship between rate of work at a heart rate of 130 beats/min ( $W_{130}$ ) kpm/min and heart volume ml. Symbols as in Fig. 2.

Fig 4 The relationship between heart volume ml and total hemoglobin content g. Symbols as in Fig. 2.



## LUNG FUNCTION STUDIES

Spirometric lung function studies were performed on the whole main series (116 patients). The ventilation capacity and also the lung volumes (with the helium dilution method) were studied. In 7 of the women, however the lung volume measurements were not satisfactory. The analyses of the values obtained for functional residual capacity, residual volume and

total lung capacity were therefore based on only 109 of the 116 patients. 12 patients who were diagnosed as having a chronic disease in addition to the bronchial asthma are reported separately (page 63).

Table 9 gives the mean values and ranges (lowest and highest values) of the data obtained on ventilation capacity and lung volumes.

Sex Age, yrs	No.	VC l	FEV <sub>1</sub> l	TV l	MMV <sub>20</sub> l/min	MMV <sub>1</sub> l/min	No.	TLC l	FRC/ TLC	RV/ TLC
Male										
60-68 mean	16	2.7	2.1	85	63	73	16	6.8	63	49
highest		5.1	3.5	71	92	108		10.6	77	59
lowest		2.0	0.7	28	31	28		4.6	51	40
54-59 mean	13	3.8	2.2	85	64	77	13	6.3	59	43
highest		4.8	3.1	71	114	121		7.8	74	65
lowest		3.0	1.3	35	35	43		5.1	43	30
30-49 mean	13	4.9	3.3	95	100	118	13	7.3	65	51
highest		6.3	5.0	96	163	210		9.3	68	43
lowest		4.0	1.9	46	46	62		5.8	44	26
Female										
60-68 mean	28	2.5	1.5	60	44	58	28	4.5	63	50
highest		3.8	2.3	80	66	92		6.8	80	73
lowest		1.4	0.6	38	25	34		2.6	47	30
50-59 mean	17	3.8	1.7	60	53	63	18	4.4	61	46
highest		3.8	2.7	86	65	119		6.7	71	68
lowest		2.0	0.9	43	38	37		3.4	47	30
30-49 mean	20	3.3	2.2	67	68	80	26	6.0	64	39
highest		3.0	3.3	81	99	124		6.9	75	65
lowest		2.7	1.1	41	33	36		3.5	37	26

Table 2. Mean values highest and lower values, concerning lung volume and ventilation capacity in different patient groups, classified according to sex and age.

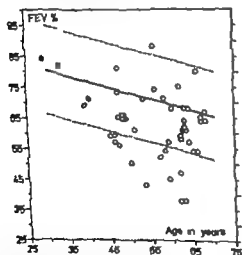


Fig. 5. FEV<sub>1</sub> % in relation to age for the males.

Central line indicates normal regression between these parameters. Interrupted lines indicate  $\pm$  twice standard error of estimate (Berglund, Birath, Bjurs, Gahrby, Kjellmer, Sandqvist and Söderholm, 1963).

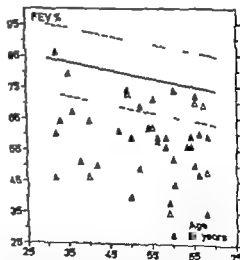


Fig. 6. FEV<sub>1</sub> % in relation to age for the males.

Central line indicates normal regression between these parameters. Interrupted lines as in Figure 5.

	VC	FEV <sub>1.0</sub>	FEV <sub>1</sub>	MVV <sub>50</sub>	MVV <sub>1</sub>	TLC	FRC/TLC	RV/TLC
VC		0.71***	0.36**	0.61***	0.59***	0.42***	-0.40**	-0.41***
FEV <sub>1.0</sub>	0.71***		0.85**	0.81**	0.79***	0.09	-0.48***	-0.33**
FEV <sub>1</sub>	0.26	0.83**		0.6***	0.66***	-0.24	-0.39***	-0.42***
MVV <sub>50</sub>	0.61***	0.81	0.6***		0.91***	0.10	-0.43***	-0.49***
MVV <sub>1</sub>	0.59***	0.79***	0.66**	0.91**		0.01	-0.44***	-0.51***
TLC	0.42***	0.09	-0.21	0.10	0.01		0.20*	0.27**
FRC/TLC	-0.40***	-0.48**	-0.39***	-0.43**	-0.44**	0.20*		0.77***
RV/TLC	-0.41	-0.52***	-0.42**	-0.40**	-0.51***	0.27**	0.77***	

Table 10 Correlation coefficients — with significance asterisks — between pairs of indices of respiratory function

The indices are expressed as ratios between observed and predicted values for each subject.

Figures 5 and 6 illustrate the position of the patients in the asthma series and regression lines for the control series with regard to FEV<sub>1</sub> and age. It is shown that the majority of the women are found between the two interrupted lines corresponding to regression line  $\pm 2$  residual SD while this is not valid for the men.

Table 10 shows the degree of agreement between pairs of different lung function tests. This agreement is expressed by means of correlation coefficients, the significance of which is demonstrated by asterisks. Very strong relationship is seen between the majority of combinations of pairs of lung function tests. The highest correlation coefficient (0.91) is obtained for MVV<sub>50</sub>—MVV<sub>1</sub>. This relationship is illustrated further by the following: for each of these two tests the 20 patients who were recorded as having the least favourable, i.e. the lowest values, were selected from the total 116. Each patient was represented by the highest value which he produced and this value is given as a percentage of the predicted value. Of the

20 patients no fewer than 14 were commonly selected for both MVV<sub>50</sub> and MVV<sub>1</sub>. This figure was compared with an expected figure — assuming that there was no relationship — of 3.5. All correlations are significant except between three pairs, i.e. TLC — FEV<sub>1.0</sub>, TLC — MVV<sub>50</sub> and TLC — MVV<sub>1</sub>.

Mean values and ranges for data obtained in different tests of ventilation capacity and lung volume

For each of the lung function tests the mean figures and ranges are given both for the entire material and for the material divided into groups according to sex and assessed degree of severity of the disease based on the total number of sickness points. The results are given both as the difference from the predicted value (mean figure and distribution) and as the mean of the individual ratios between observed and predicted values expressed in per cent. The predicted values are obtained according to Table 1 (for method see page 28).

### Vital capacity (VC)

As shown in Table 11 the mean value for the vital capacity expressed as a percentage of the predicted value was 85.0 for the men and 87.1 for the women. The mean difference between the observed and the predicted value was -0.7 litre for the men and -0.4 litre for the women. This difference deviates significantly from zero both for the men and for the women (\*).

The patients were classified into groups according to the estimated total number of sickness points since the onset of the asthma, and the quotient between the observed value and the predicted value (expressed in per cent) was obtained for each individual. For

those with fewer than 1000 points (group A) this mean percentage value was 89.6. For those who had attained 1000 but not 2000 points (group B) this value was 85.9 %, and for those who had attained 2000 or more (group C) 85.8 %.

The mean difference between group A and groups B and C combined, and also between group B and group C, was then calculated. The difference between A and B+C was significant (\*) when the relative frequency of cases with a positive difference by  $\chi^2$  test was compared (cf page 159). No significant difference was found between B and C.

Patients according to sex and sickness points	Difference between observed VC and predicted use (litres)										Difference from the predicted value (litres) men	Percentage of predicted value mean
	%	$\geq +0.7$	+0.3	+0.2	-0.3	-0.7	-1.1	-1.5	-1.9	$\leq -2.3$		
Male	42	0	3	9	11	6	7	3	2	1	-0.7***	85.0
Female	71	1	3	20	27	18	4	1	0	0	-0.4***	87.1
Total	116	1	6	29	38	24	11	4	2	1	-0.5***	86.3
Group A												
Male	12	0	1	3	3	2	1	1	0	1	-0.7*	86.1
Female	26	0	0	14	8	3	1	0	0	0	-0.5***	87.2
Total	38	0	1	17	11	5	2	1	0	1	-0.4***	89.6
Group B												
Male	16	0	1	4	3	1	0	1	1	0	-0.9***	82.1
Female	25	0	2	3	9	9	2	0	0	0	-0.5***	85.0
Total	41	0	3	7	12	10	2	1	1	0	-0.7***	83.9
Group C												
Male	14	0	2	2	5	3	0	1	1	0	-0.9*	87.3
Female	23	1	1	3	10	6	1	1	0	0	-0.5***	84.7
Total	37	1	3	5	15	9	1	2	1	0	-0.8***	85.8

Table 11. Vital capacity (VC) in different patient groups, classified according to sex and estimated number of "sickness points" since the manifestation of the bronchial asthma. The differences between the individual values of VC and the individual predicted values (predicted according to sex, age and height) are given. The significance of differences from the predicted value is stated with significance asterisks. Means of the individual ratios between observed and predicted values expressed in per cent are also given.

# Forced expiratory volume in one second (FEV<sub>1.0</sub>)

As may be seen in Table 12 the mean value for the forced expiratory volume in one second given as a percentage of the predicted value, was 70.0 for the men and 70.8 for the women. The mean difference between the observed value and predicted value was -1.0 litre for the men and -0.7 litre for the women. This difference deviates significantly from zero for both the men and the women (\*\*\*)

The patients were classified into groups according to the estimated total number of sickness points since the onset of the asthma and the quotient between the observed and the predic

ted value (expressed in per cent) was obtained for each individual. For those with fewer than 1000 points (group A) this mean percentage value was 78.6. For those who had attained 1000 but not 2000 points (group B) a value of 67.5 % was obtained, and for those who had attained 2000 points or more (group C) 65.5 %.

The mean difference between group A and groups B and C combined, and also between group B and group C, were then calculated. The difference between A and B+C was found to be significant (\*\*). There was no significant difference between B and C. (Significance test according to point 2 on page 169)

Patients according to sex and sickness points	Difference between observed FEV <sub>1.0</sub> and predicted value (litres)										Difference from the predicted value (litres) mean	Percentage of predicted value mean
	No	≥ +0.7	+0.3 +0.6	+0.3 -0.1	-0.3 -0.6	-0.7 -1.0	-1.1 -1.4	-1.5 -1.8	-1.9 -2.2	≤ -2.3		
Male	42	1	0	5	8	12	5	4	4	3	-1.0***	70.0
Female	74	0	0	9	21	25	16	3	0	0	-0.7**	70.8
Total	116	1	0	14	29	37	21	7	4	3	-0.9***	70.5
Group A												
Male	12	1	0	3	2	2	1	1	1	1	-0.5*	77.3
Female	20	0	0	5	11	9	1	0	0	0	-0.5***	79.2
Total	32	1	0	8	13	11	2	1	1	1	-0.6***	78.6
Group B												
Male	16	0	0	1	3	6	1	3	1	1	-1.1***	67.3
Female	25	0	0	2	5	9	7	2	0	0	-0.9***	67.6
Total	41	0	0	3	8	15	8	5	1	1	-1.0***	67.5
Group C												
Male	14	0	0	1	3	3	3	0	2	1	-1.1***	66.9
Female	23	0	0	2	5	7	8	1	0	0	-0.9*	64.7
Total	37	0	0	3	8	11	11	1	2	1	-1.0***	65.5

Table 12. Forced expiratory volume in one second (FEV<sub>1.0</sub>) in different patient groups classified according to sex and estimated number of "sickness points" since the manifestation of the bronchial asthma. The differences between the individual values of FEV<sub>1.0</sub> and the individual predicted values (prediction according to sex, age and height) are given. The significance of difference from the predicted value is stated with significance asterisks. Means of the individual ratio between observed and predicted values expressed in per cent are also given.

Forced expiratory volume in one second ( $FEV_{1s}$ ) expressed as a percentage of vital capacity (VC) i.e.  $FEV_x$

Table 13 shows that the mean value for the forced expiratory volume in one second ( $FEV_{1s}$ ) expressed as a percentage of the vital capacity ( $FEV_x$ ) was 79.8 % of the predicted value for the men and 79.6 % for the women. The mean difference between the observed and the predicted value was -14.2 per cent units for the men and -15.9 per cent units for the women. This difference deviated significantly from zero for both the men and the women ( )

The patients were classified into groups according to the estimated total number of sickness points, and the

quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient. For those with fewer than 1000 sickness points (group A) this mean percentage value was 86.3. For those who had attained 1000 but not 2000 points (group B) a value of 78.0 % was obtained, and for those who had attained 2000 points or more (group C) 74.7 %

The mean difference between group A and groups B and C combined, and also between group B and group C, were than calculated. The difference between A and B+C was found to be significant (\*). No significant difference was found between groups B and C.

Patients according to sex and sickness points	Difference between observed $FEV_x$ and predicted value (per cent units)										Difference from the predicted value (per cent units) mean	Percentage of predicted value mean
	No.	$\geq +11$	+10 + 6	+8 - 8	- 6 -10	-11 -18	-16 -20	-21 -25	-26 -30	$\leq -31$		
Male	42	6	1	9	5	11	5	2	2	6	-14.2***	79.8
Female	74	0	1	11	15	14	10	9	8	9	-15.9***	79.6
Total	116	6	2	20	20	25	15	11	8	15	-15.3***	79.7
Group A												
Male	11	0	0	0	1	2	0	1	1	1	- 8.8*	86.7
Female	26	0	1	5	5	5	5	3	0	0	-16.8***	86.1
Total	37	0	1	5	6	7	5	4	1	1	-10.3***	86.3
Group B												
Male	16	0	1	2	4	2	2	1	2	1	-14.3**	79.8
Female	25	0	0	2	5	5	3	4	4	2	-18.1***	78.9
Total	41	0	1	4	9	7	5	5	6	3	-16.5***	78.9
Group C												
Male	15	0	0	1	0	7	2	0	0	4	-18.1***	74.0
Female	23	0	0	3	5	4	1	2	1	7	-19.3***	75.1
Total	37	0	0	4	5	11	3	2	1	11	-18.8***	74.7

Table 13. Forced expiratory volume in one second ( $FEV_{1s}$ ) expressed as percentage of vital capacity (VC),  $FEV_x$ , in different patient groups, classified according to sex and estimated number of sickness points since the manifestation of the bronchial asthma. The differences between the individual values of  $FEV_{1s}$  and the individual predicted values (prediction according to sex and age) are given. The significant difference from the predicted value is noted with significance materials. Means of the individual values between observed and predicted values, expressed in per cent are also given.



*Maximum voluntary ventilation determined at a fixed frequency of 40 breaths per minute ( $MVV_{40}$ )*

As may be seen in Table 14 the mean value for the maximal voluntary ventilation at a fixed frequency of 40 breaths per minute ( $MVV_{40}$ ) was 60.3 % of the predicted value for the men and 61.3 % for the women. The mean difference between the observed and the predicted value was -48.0 litres for the men and -33.6 litres for the women. This difference deviated significantly from zero both for the men and the women (\*\*\*).

The patients were classified into groups according to the estimated total number of sickness points and the

quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient. For those with fewer than 1000 points (group A) this mean value was 68.4 %. For those who had attained 1000 but not 2000 points (group B) a value of 56.7 % was obtained, and for those who had attained 2000 points or more (group C) 57.9 %.

The mean difference between group A and groups B and C combined, and also between group B and group C, were then calculated. The difference between A and B+C was found to be significant (\*\*). No significant difference was found between B and C.

Table is according to sex and sickness points	Difference between observed $MVV_{40}$ and predicted value (litres)										Difference from the predicted value (litres) men	Percent age of predicted value mean
	No	$\geq +1$	+20 +11	+10 -10	-11 -20	-21 -30	-31 -40	-41 -50	-51 -60	$\leq -61$		
Male	12	0	0	3	3	5	3	7	0	12	-48.0***	60.3
Female	4	0	0	0	8	12	12	20	16	6	-33.0***	61.3
Total	116	0	0	9	14	17	23	23	16	18	-38.8***	60.0
Group A												
Male	12	0	0	3	2	0	0	3	1	3	-37.7**	69.2
Female	26	0	0	4	6	4	8	5	0	0	-28.0***	68.0
Total	38	0	0	7	7	4	8	8	1	3	-31.1***	68.4
Group B												
Male	10	0	0	0	1	1	2	3	5	4	-33.5**	54.9
Female	20	0	0	0	4	3	8	7	3	0	-37.0***	57.6
Total	41	0	0	0	5	4	10	10	8	4	-43.4**	56.7
Group C												
Male	14	0	0	0	0	4	1	1	3	5	-50.6***	58.7
Female	23	0	0	2	2	5	4	6	3	1	-36.4***	57.1
Total	37	0	0	2	2	9	5	7	6	6	-41.8***	59

Table 14. *Maximum voluntary ventilation determined at a fixed frequency of 40 breaths per minute ( $MVV_{40}$ ) in different patient groups classified according to sex and estimated number of sickness points since the manifestation of the bronchial asthma. The difference between the individual value of  $MVV_{40}$  and the individual predicted values (predicted according to sex and age) are given. The significance of difference from the predicted value is indicated with significance asterisks. Means of the individual ratios between observed and predicted values expressed in per cent are also given.*

*Maximum voluntary ventilation determined at a frequency chosen by the patient (MVV<sub>p</sub>)*

As may be seen in Table 15 the mean value for the maximum voluntary ventilation determined at a frequency chosen by the patient was 68.0 % of the predicted value for the men and 59.8 % for the women. The mean difference between the observed and the predicted value was -62.0 litres for the men and -12.0 litres for the women. This difference deviated significantly from zero both for the men and for the women ( \*\* )

The patients were classified into groups according to the estimated total number of sickness points since the

onset of the asthma, and the quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient. For those with fewer than 1000 points (group A) this mean value was 68.1 %. For those who had attained 1000 but not 2000 points (group B) a value of 51.1 % was obtained, and for those who had attained 2000 points or more (group C) 55.0 %.

The mean difference between group A and groups B and C combined, and also between group B and group C, were then calculated. The difference between A and B+C was found to be significant ( \*\* ) No significant difference was found between B and C.

Patients according to sex and sickness points	Differences between observed MVV <sub>p</sub> and predicted value (litres)										Difference from the predicted value (litres) mean	Percent of predicted value mean
	No.	+21	+20	+10	-11	-21	-31	-41	-51	≤ -61		
Male	42	1	0	0	0	4	8	4	4	23	-62.0***	68.0
Female	74	0	1	5	5	15	11	13	17	19	-12.0***	59.8
Total	116	1	1	5	5	14	17	17	21	35	-19.2***	60.2
Group A												
Male	13	1	0	0	0	2	2	1	1	6	-18.5**	67.3
Female	26	0	1	2	2	4	5	4	5	1	-23.0***	62.5
Total	39	1	1	2	2	6	7	5	6	7	-20.7***	62.1
Group B												
Male	18	0	0	0	0	1	2	2	0	11	-60.1***	52.6
Female	25	0	0	0	1	4	2	7	6	6	-47.8***	53.0
Total	43	0	0	0	1	5	4	9	6	17	-54.2***	51.1
Group C												
Male	33	0	0	0	0	1	2	1	3	7	-63.2***	56.3
Female	23	0	0	2	1	2	4	2	7	5	-46.0***	55.3
Total	57	0	0	2	1	3	6	3	10	12	-54.2***	55.8

Table 15. Maximum voluntary ventilation determined at frequency chosen by the patient (MVV<sub>p</sub>) in different patient groups classified according to sex and estimated number of sickness points since the manifestation of the bronchial asthma. The difference between the individual value of MVV<sub>p</sub> and the individual predicted values (prediction according to sex, age and height) is given. The significance of difference from the predicted value is stated with asterisks. Means of the individual ratios between observed and predicted values expressed in per cent, are also given.

*Maximum voluntary ventilation determined at a fixed frequency of 40 breaths per minute (MVV<sub>40</sub>)*

As may be seen in Table 14 the mean value for the maximal voluntary ventilation at a fixed frequency of 40 breaths per minute (MVV<sub>40</sub>) was 60.3 % of the predicted value for the men and 61.3 % for the women. The mean difference between the observed and the predicted value was -48.0 litres for the men and -33.6 litres for the women. This difference deviated significantly from zero both for the men and the women (\*\*\*)

The patients were classified into groups according to the estimated total number of sickness points, and the

quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient. For those with fewer than 1000 points (group A) this mean value was 63.4 %. For those who had attained 1000 but not 2000 points (group B) a value of 56.7 % was obtained, and for those who had attained 2000 points or more (group C) 57.0 %.

The mean difference between group A and groups B and C combined, and also between group B and group C, were then calculated. The difference between A and B+C was found to be significant (\*\*). No significant difference was found between B and C.

Patients according to sex and sickness points	Difference between observed MVV <sub>40</sub> and predicted value (litres)										Difference from the predicted value (litres) mean	Percent age of predicted value mean
	No	-2 +1	+0 +11	+10 -10	-11 -20	-21 -30	-31 -40	-41 -50	-51 -60	-61		
Male	42	0	0	3	3	5	3	7	9	12	-48.0***	60.3
Female	74	0	0	5	11	12	20	18	6	1	-33.6***	61.3
Total	116	0	0	8	14	17	23	25	15	13	-38.8***	60.9
Group A												
Male	12	0	0	3	2	0	0	3	1	3	-37.7**	69.2
Female	20	0	0	4	5	4	8	3	0	0	-28.0***	68.0
Total	32	0	0	7	7	4	8	6	1	3	-31.1***	68.4
Group B												
Male	16	0	0	0	1	1	2	3	5	4	-53.5**	54.9
Female	25	0	0	0	4	3	8	7	3	0	-37.0***	57.8
Total	41	0	0	0	5	4	10	10	8	4	-43.4***	56.4
Group C												
Male	14	0	0	0	0	4	1	1	3	5	-50.6**	58.7
Female	23	0	0	2	2	5	4	6	3	1	-36.1**	57.4
Total	37	0	0	2	2	9	5	7	6	6	-41.8***	57.0

Table 14. Maximum voluntary ventilation determined at a fixed frequency of 40 breaths per minute (MVV<sub>40</sub>) in different patient groups classified according to sex and estimated number of sickness points since the manifestation of the bronchial asthma. The difference between the individual values of MVV<sub>40</sub> and the individual predicted values (predicted according to sex and age) are given. The significance of difference from the predicted value is tested with significance asterisks. Means of the individual ratios between observed and predicted values expressed in per cent are also given.

*Maximum voluntary ventilation determined at a frequency chosen by the patient (MVV<sub>1</sub>)*

As may be seen in Table 15 the mean value for the maximum voluntary ventilation determined at a frequency chosen by the patient was 58.0 % of the predicted value for the men and 59.8 % for the women. The mean difference between the observed and the predicted value was -62.0 litres for the men and -42.0 litres for the women. This difference deviated significantly from zero both for the men and for the women (\*\*)

The patients were classified into groups according to the estimated total number of sickness points since the

onset of the asthma, and the quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient. For those with fewer than 1000 points (group A) this mean value was 68.1 %. For those who had attained 1000 but not 2000 points (group B) a value of 54.1 % was obtained, and for those who had attained 2000 points or more (group C) 55.6 %.

The mean difference between group A and groups B and C combined, and also between group II and group C, were then calculated. The difference between A and B+C was found to be significant (\*) No significant difference was found between B and C.

Patients according to sex and sickness points	Difference between observed MVV <sub>1</sub> and predicted value (litres)										Difference from the predicted value (litres) mean	Percent of predicted value
	No.	≥ +21	+20 +11	+10 +10	-11 -20	-21 -30	-31 -40	-41 -50	-51 -60	≤ -61		
Male	42	1	0	0	0	4	6	4	4	23	-62.0***	58.0
Female	74	0	1	5	8	10	11	13	17	12	-42.0***	59.8
Total	116	1	1	5	8	14	17	17	21	35	-52.7***	58.9
Group A												
Male	12	1	0	0	0	2	2	1	1	6	-48.5**	67.3
Female	26	0	1	3	2	4	5	4	5	1	-33.0***	68.5
Total	38	1	1	3	2	6	7	5	6	7	-37.9***	68.1
Group B												
Male	16	0	0	0	0	1	2	2	0	11	-62.1***	59.8
Female	25	0	0	0	1	4	2	7	5	6	-47.8***	61.0
Total	41	0	0	0	1	5	4	9	5	17	-54.2***	54.1
Group C												
Male	14	0	0	0	0	1	2	1	3	7	-63.2***	56.3
Female	23	0	0	3	1	2	1	2	7	6	-46.0***	65.2
Total	37	0	0	3	1	3	3	3	10	13	-53.2***	55.6

Table 15. Maximum voluntary ventilation determined at frequency chosen by the patient (MVV<sub>1</sub>) in different patient groups classified according to sex and estimated number of sickness points since the manifestation of the bronchial asthma. The difference between the individual value of MVV<sub>1</sub> and the individual predicted values (predicted according to sex, age and height) are given. The significance of difference from the predicted value is stated with significance materials. Means of the individual ratios between observed and predicted values expressed in per cent, are also given.

### Total lung capacity (TLC)

Table 16 shows that the mean value for the total lung capacity was 94.4 % of the estimated normal value for the men and 93.6 % for the women. The mean difference between the observed and the predicted value was -0.4 litre for the men and -0.3 litre for the women. This difference deviated significantly from zero both for the men and for the women (\*).

The patients were classified into groups according to the total number of sickness points since the onset of the asthma and the quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient. For those with fewer

than 1000 points (group A) this mean percentage value was 92.5. For those who had attained 1000 but not 2000 points (group B) this value was 94.2 %, and for those who had attained 2000 points or more (group C) 95.0 %.

The mean difference between group A and groups B and C combined, was then calculated. This difference, however, was not significant.

### Functional residual capacity (FRC) in relation to total lung capacity (TLC)

Table 17 shows that the mean value for the functional residual capacity in relation to the total lung capacity was 111.3 % of the predicted value

Patients according to sex and sickness points	Difference between observed TLC and predicted value (litres)										Difference from the predicted value (litres) mean	Percent age of predicted value mean
	No	≥ +1.5	+1.4 +1.1	+1.0 +0.7	+0.6 +0.3	+0.3 -0.3	-0.3 -0.6	-0.7 -1.0	-1.1 -1.4	≤ -1.5		
Male	42	2	1	1	6	8	7	6	6	6	-0.4	94.4
Female	67	0	0	6	11	15	10	17	4	4	-0.3*	93.6
Total	109	2	1	7	17	23	17	23	10	9	-0.4***	93.9
Group A												
Male	12	0	0	0	3	1	2	2	3	1	-0.6	91.8
Female	24	0	0	1	4	5	5	6	2	1	-0.4	92.9
Total	36	0	0	1	7	6	7	8	5	2	-0.5	92.5
Group B												
Male	16	1	0	0	2	3	3	3	1	3	-0.4	93.5
Female	21	0	0	2	2	7	2	7	1	0	-0.3	94.9
Total	37	1	0	2	4	10	5	10	2	3	-0.3*	94.2
Group C												
Male	14	1	1	1	1	4	2	1	2	1	-0.2	97.7
Female	22	0	0	3	5	3	3	4	1	3	-0.3	93.3
Total	36	1	1	4	6	7	5	5	3	4	-0.3	95.0

Table 16. Total lung capacity (TLC) in different patient groups, classified according to sex and estimated number of "sickness points" since the manifestation of the bronchial asthma. The differences between the individual values of TLC and the individual predicted value (predicted according to sex, age, height and weight) are given. The significance of difference from the predicted value is stated with significance asterisks. Means of the individual ratios between observed and predicted values, expressed in per cent, are also given.

for the men and 121.6% for the women. The mean difference between the observed and the predicted value was +0.1 per cent units for the men and +10.6 per cent units for the women. This difference deviated significantly from zero both for the men and for the women ( \* )

The patients were classified into groups according to the estimated total number of sickness points since the onset of the asthma, and the quotient between the observed and the predicted value (expressed in per cent) was obtained for each patient. For those with fewer than 1000 points (group A) this mean percentage value was 106.4 for

the men and 114.8 for the women. For the men who had attained 1000 but not 2000 points (group B) a value of 112.1 % was obtained, and for the men who had attained 2000 or more (group C) 114.7 %. The corresponding values for the women were 124.4 % and 126.3 %.

The mean difference between group A and groups II and C combined, and also between group B and group C, were then calculated. For the men the difference between A and B+C was not significant. For the women the corresponding difference was significant ( \* ) %o significant difference was found between II and C, either for the men or the women.

Patients according to sex and sickness points	Difference between observed FRC/TLC and predicted value (per cent units)										Difference from the predicted value (per cent units) mean	Percent age of predicted value mean
	%	≥ +31	+30 +25	+25 +21	+20 +16	+15 +11	+10 + 6	+5 -4	-5 -10	≤ -11		
Male	43	0	0	0	0	7	10	16	2	0	+ 8.1***	111.3
Female	57	0	2	2	10	17	13	18	2	0	+10.6***	121.6
Total	109	0	2	2	10	24	22	24	2	0	+ 8.9***	117.6
Group A												
Male	12	0	0	0	1	2	2	4	2	0	+ 2.4	106.4
Female	21	0	1	0	2	5	3	10	1	0	+ 7.3***	114.8
Total	36	0	1	0	2	7	5	14	2	0	+ 6.0***	112.0
Group B												
Male	10	0	0	0	2	4	2	6	1	0	+ 6.6**	112.1
Female	21	0	1	2	3	6	4	4	1	0	+12.0***	124.4
Total	31	0	1	2	5	10	7	10	2	0	+ 8.7***	119.1
Group C												
Male	14	0	0	0	3	1	4	6	0	0	+ 7.9**	114.7
Female	23	0	1	2	5	6	3	4	0	0	+12.0***	126.3
Total	38	0	1	2	8	7	7	10	0	0	+11.0***	121.7

Table 17. Functional residual capacity (FRC) in relation to total lung capacity (TLC) in different patient groups, classified according to sex and estimated number of sickness points since the manifestation of the bronchial asthma. The differences between the individual values of FRC/TLC and the individual predicted values (prediction according to sex, age and weight) are given. The significance of difference from the predicted value is stated with significance symbols. Means of the individual values between observed and predicted values, expressed in per cent are also given.

# Residual volume (RV) in relation to total lung capacity (TLC)

As may be seen in Table 18 the mean value for the residual volume in relation to the total lung capacity was 131.0 % of the predicted value for the men and 147.0 % for the women. The mean difference between the observed and the predicted value was +11.7 per cent units for the men and +14.0 per cent units for the women. This difference deviated significantly from zero both for the men and the women (\*\*\*)

The patients were classified into groups according to the estimated total number of sickness points since the onset of the asthma and the quotient between the observed value and the predicted value (expressed in per cent) was calculated for each patient. For

those with fewer than 1000 points (group A) this mean percentage figure was 132.0 for the men and 131.5 for the women. For those men who had attained 1000 but not 2000 points (group B) a value of 135.5 % was obtained, and for those who had attained 2000 points or more (group C) 144.7 %. The corresponding values for the women were 153.4 % and 153.0 %.

The mean difference between group A and groups B and C combined, and also between group B and group C, were then calculated. For the men the difference between A and B+C was not significant. For the women the corresponding difference was significant (\*). No significant difference was found between B and C either for the men or the women.

Patient according to sex and sickness points	Difference between observed RV/TLC and predicted value (per cent units)										Difference from the predicted value (per cent units) mean	Percent age / predicted value mean
	No	+31	+30	+29	+28	+27	+26	+25	+24	+23		
Male	42	1	3	3	2	10	14	0	0	0	+11.7***	131.8
Female	67	4	2	11	9	16	13	13	0	0	+14.6***	147.9
Total	109	5	5	14	11	26	27	21	0	0	+13.5**	143.9
Group A												
Male	12	0	1	1	1	3	1	5	0	0	+9.9**	132.0
Female	21	0	0	4	3	4	8	5	0	0	+11.5***	131.5
Total	33	0	1	5	4	7	9	10	0	0	+11.0**	133.7
Group B												
Male	16	0	1	2	0	3	6	4	0	0	+12.3**	135.5
Female	31	2	0	4	2	7	3	3	0	0	+16.1***	153.4
Total	37	2	1	6	2	10	9	7	0	0	+14.0***	145.7
Group C												
Male	14	1	1	0	1	4	7	0	0	0	+12.0***	144
Female	22	2	2	3	4	5	2	4	0	0	+16.6***	153.0
Total	36	3	3	3	5	9	9	4	0	0	+15.4***	150.3

Table 18. Residual volume (RV) in relation to total lung capacity (TLC) in different patient groups classified according to sex and estimated number of "sickness points" since the manifestation of the bronchial asthma. The differences between the individual value of RV/TLC and the individual predicted values (prediction according to sex, age, height and weight) are given. The significance of difference from the predicted value is noted with significance asterisks. Means of the individual ratios between observed and predicted values expressed in per cent are also given.

In summary it may be said that for each of the different indices of respiratory function the mean observed value for the patients in the asthma series differed significantly from the predicted value. The greatest percentage difference concerned the maximum voluntary ventilation, the forced expiratory volume per one second, and the ratio of residual volume to total lung capacity. The smallest difference concerned the total lung capacity and vital capacity.

When the patients were classified into groups according to the estimated degree of severity of the disease—based on the total number of sickness points—significant differences were found for several of the function variables between groups or combination of groups. The differences with the highest degree of significance were found in the tests indicating ventilation capacity. These differences were shown between the mean value for the group in which none of the patients had attained 1000 sickness points, and that for the group in which every patient had attained 1000 points or more. The only function variable for which a significant difference between the groups was not shown was the total lung capacity.

With regard to the ratios of the functional residual capacity and residual volume to total lung capacity the difference between the observed and the predicted values—calculated from the control material—was on an average somewhat greater for the women than for the men. As regards the other function variables, no sex

difference of any notable magnitude was found.

Wide ranges were found for each of the function variables both when the material was regarded as a whole and when it was divided into groups according to the estimated degree of severity of the disease.

### Analysis of the relationship between some case history factors and different indices of respiratory function

An attempt was made to analyse the influence on the respiratory function of different case history factors of especial significance in bronchial asthma.

Table 19 shows the mean values of the respiratory function variables for the different patient groups distributed according to the case history factors. The latter factors comprised sex, age, estimated total number of sickness points, duration, age at onset, estimated total number of attack hours, estimated number of sickness points during the period 1957–1961 and the number of admissions to hospital on account of asthma. As seen in the table considerable differences were found. A more detailed significance analysis of the relationship between each lung function variable and each of the eight case history factors is given in Table 20.

Table 20 shows the degree of correlation of the case history factors with the lung function variables. Disregarding sex and age at onset, all of the factors are significantly correlated



# Residual volume (RV) in relation to total lung capacity (TLC)

As may be seen in Table 18 the mean value for the residual volume in relation to the total lung capacity was 137.0 % of the predicted value for the men and 147.0 % for the women. The mean difference between the observed and the predicted value was +11.7 per cent units for the men and +14.6 per cent units for the women. This difference deviated significantly from zero both for the men and the women (\*\*\*)

The patients were classified into groups according to the estimated total number of sickness points since the onset of the asthma and the quotient between the observed value and the predicted value (expressed in per cent) was calculated for each patient. For

those with fewer than 1000 points (group A) this mean percentage figure was 132.0 for the men and 131.3 for the women. For those men who had attained 1000 but not 2000 points (group B) a value of 135.5 % was obtained, and for those who had attained 2000 points or more (group C) 144.7 %. The corresponding values for the women were 153.4 % and 153.0 %.

The mean difference between group A and groups B and C combined, and also between group B and group C, were then calculated. For the men the difference between A and B+C was not significant. For the women the corresponding difference was significant (\*). No significant difference was found between B and C either for the men or the women.

Patients according to sex and sickness points	Difference between observed RV/TLC and predicted value (per cent units)										Difference from the predicted value (per cent units) mean	Percent age of predicted value mean
	No	≥ +31	+30 to +20	+20 to +11	+10 to +6	+5 to +1	0 to -5	-6 to -10	≤ -11			
Male	43	1	3	3	2	10	14	9	0	0	+11.7***	137.0
Female	67	4	3	11	9	16	13	12	0	0	+14.0***	147.9
Total	109	5	6	14	11	26	27	21	0	0	+13.5**	143.0
Group A												
Male	12	0	1	1	1	3	1	5	0	0	+9.9*	132.0
Female	21	0	0	4	3	4	8	5	0	0	+11.5**	131.3
Total	33	0	1	5	4	7	9	10	0	0	+11.0***	133.7
Group B												
Male	16	0	1	2	0	3	6	4	0	0	+11.3	135.5
Female	21	2	0	4	2	7	3	3	0	0	+16.1***	153.4
Total	37	2	1	6	2	10	9	7	0	0	+14.0***	144.7
Group C												
Male	14	1	1	0	1	4	7	0	0	0	+12.6***	141.7
Female	22	2	2	3	4	5	2	4	0	0	+16.6***	153.0
Total	36	3	3	3	5	9	9	4	0	0	+15.1***	150.3

Table 18. Residual volume (RV) in relation to total lung capacity (TLC) in different patient groups classified according to sex and estimated number of sickness points since the manifestation of the bronchial asthma. The differences between the individual value of RV/TLC and the individual predicted values (prediction according to sex, age, height and weight) are given. The significance of difference from the predicted value is stated with significance marks. Means of the individual ratios between observed and predicted values expressed in per cent are also given.

In summary it may be said that for each of the different indices of respiratory function the mean observed value for the patients in the asthma series differed significantly from the predicted value. The greatest percentage difference concerned the maximum voluntary ventilation, the forced expiratory volume per one second, and the ratio of residual volume to total lung capacity. The smallest difference concerned the total lung capacity and vital capacity.

When the patients were classified into groups according to the estimated degree of severity of the disease—based on the total number of sickness points—significant differences were found for several of the function variables between groups or combination of groups. The differences with the highest degree of significance were found in the tests indicating ventilation capacity. These differences were shown between the mean value for the group in which none of the patients had attained 1000 sickness points, and that for the group in which every patient had attained 1000 points or more. The only function variable for which a significant difference between the groups was not shown was the total lung capacity.

With regard to the ratios of the functional residual capacity and residual volume to total lung capacity the difference between the observed and the predicted values—calculated from the control material—was on an average somewhat greater for the women than for the men. As regards the other function variables no sex

difference of any notable magnitude was found.

Wide ranges were found for each of the function variables both when the material was regarded as a whole and when it was divided into groups according to the estimated degree of severity of the disease.

### Analysis of the relationship between some case history factors and different indices of respiratory function

An attempt was made to analyse the influence on the respiratory function of different case history factors of especial significance in bronchial asthma.

Table 19 shows the mean values of the respiratory function variables for the different patient groups distributed according to the case-history factors. The latter factors comprised sex, age, estimated total number of sickness points, duration (age at onset, estimated total number of attack hours, estimated number of sickness points during the period 1957–1961) and the number of admissions to hospital on account of asthma. As seen in the table considerable differences were found. A more detailed significance analysis of the relationship between each lung function variable and each of the eight case-history factors is given in Table 20.

Table 20 shows the degree of correlation of the case history factors with the lung function variables. With regard to sex and age at onset, all of the factors are significantly correlated

Table 19 Percentage mean of the ratios between observed and predicted values for different indices of the respiratory function.

Quantification as described on page 41. The individual value is formed as a ratio between the observed individual values and the predicted values multiplied by 100.

No. = 116, except for attack hours	No. = 113 and sickness points 1957-61	No. = 115
No. = 109 except for attack hours	No. = 106 and sickness points 1957-61	No. = 103

with at least two of these variables. The large number of significant correlations should be regarded in the light of the fact that the eight factors are themselves mutually correlated (see Table 7) and also that there is mutual correlation between the lung function variables (see Table 10).

Since the case-history factors show a high degree of mutual correlation, it is difficult to interpret the significance coefficients shown in Table 20. It may be considered that the relationships between certain factors and certain lung function variables are only apparent. In order to better evaluate the influence of the factors, a regression analysis was performed. The lung function variables were regarded as dependent variables and the eight case-history factors as independent. The relationship may be expressed as the following linear equation

$$y = a + b_1 X_1 + b_2 X_2 + \dots + b_8 X_8$$

where  $y$  is lung function variable

$$\frac{\text{observed value}}{\text{predicted value}} \times 100$$

$a, b_1, b_2, b_3, \dots, b_8$  the coefficients to be evaluated,

and  $X_1, X_2, X_3, \dots, X_8$  the factors referred to in Table 10. Quantification was performed as shown by this table and also on page 41.

For the calculation of these coefficients there were 116 individual observations comprising data for  $X_1, X_2, X_3,$

$X_4$ , and also the lung function variables (for TLC, FRC/TLC and RV/TLC, however data were only obtained for 109 patients).

By means of this regression analysis the coefficients  $b_1, b_2, b_3, b_4$  were estimated. The numerical results of the analysis are given in Table 21.

As a complement to Table 21 a similar regression analysis was performed

		VC %	FEV <sub>1.0</sub> %	FEV <sub>2.0</sub> %	MVV <sub>0</sub> %	MVV <sub>1</sub> %	TLC %	FRC/TLC %	RV/TLC %
sex	$X_1$	-0.07	-0.01	0.06	-0.03	-0.04	0.03	-0.30**	-0.17
age	$X_2$	0.38***	0.39*	0.63	0.32***	0.31***	0.06	-0.21	-0.08
Total such nose points	$X_3$	-0.11	-0.20**	-0.36**	-0.24	-0.26**	0.07	0.24	0.21
Duration	$X_4$	-0.09	-0.28**	-0.23***	-0.18	-0.22*	0.17	0.19*	0.18
Age 1 onset	$X_5$	-0.20**	-0.01	0.10	-0.14	-0.12	-0.10	0.16	0.00
Attack hours	$X_6$	-0.04	-0.16	-0.21	-0.20*	-0.20*	-0.01	0.10	-0.03
Backrest points 1937-61	$X_7$	-0.18	-0.28**	-0.28**	-0.35***	-0.30**	-0.00	0.14	0.12
Admission to hospital	$X_8$	-0.15	-0.18	-0.17	-0.19*	-0.21	-0.10	0.07	0.00

Table 20 Correlation coefficients — with significance asterisks — between case history factors and indices of respiratory function.

Quantification of factors shown in Table 10 and described on page 41.

Groups according to case history factors	Quantification	VC					TLC		
		No	% of pred. Y1	% of pred. Y2	% of pred. Y3	% of pred. Y4	% of pred. Y5	No.	% of pred. Y6
Sex									
Female	X <sub>1</sub> =0	74	87.1	70.8	79.6	61.3	59.8	67	93.6
Male	X <sub>1</sub> =1	42	85.0	70.0	79.8	60.9	58.0	42	94.4
Age									
60-69 yrs.	X <sub>2</sub> =1	44	80.5	68.0	79.9	55.7	52.6	42	91.3
50-59 yrs.	X <sub>2</sub> =2	30	85.8	66.5	76.2	57.8	56.4	28	90.6
30-49 yrs.	X <sub>2</sub> =3	42	92.7	76.0	81.9	68.9	68.1	39	96.1
Total sickness points									
<1000 p	X <sub>3</sub> =1	38	89.6	78.6	86.3	68.4	68.1	36	92.5
1000-1999 p	X <sub>3</sub> =2	41	83.9	67.5	78.0	56.7	54.1	37	94.2
≥2000 p	X <sub>3</sub> =3	37	85.8	65.5	74.7	57.9	55.6	36	96.0
Duration									
<15 yrs.	X <sub>4</sub> =1	32	88.9	77.9	87.0	63.7	62.9	30	91.7
15-24 yrs.	X <sub>4</sub> =2	51	85.3	70.0	79.2	62.4	60.5	49	94.0
≥25 yrs.	X <sub>4</sub> =3	33	85.4	64.0	73.4	55.9	53.6	30	96.1
Age at onset									
≤16 yrs.	X <sub>5</sub> =1	22	92.9	76.6	81.6	69.6	67.6	21	98.3
17-30 yrs.	X <sub>5</sub> =2	51	86.7	66.7	76.0	58.1	56.5	47	92.3
≥31 yrs.	X <sub>5</sub> =3	43	82.5	71.8	83.3	59.7	58.3	41	93.6
Attack hours									
1-490 hrs.	X <sub>6</sub> =1	37	87.7	74.4	82.9	66.0	64.2	35	94.1
500-2499 hrs.	X <sub>6</sub> =2	32	83.3	72.0	81.8	59.9	60.3	29	93.9
≥2500 hrs.	X <sub>6</sub> =3	44	85.8	66.1	75.2	57.1	54.1	42	93.8
Sickness points 1957-61									
<50 p.	X <sub>7</sub> =1	70	87.4	73.6	82.5	65.4	67.3	65	94.4
50-99 p.	X <sub>7</sub> =2	27	86.1	67.5	76.4	54.4	53.2	25	94.7
≥100 p.	X <sub>7</sub> =3	18	81.7	61.1	72.3	51.6	49.6	18	90.8
Admissions to hospital									
1-4 times	X <sub>8</sub> =1	81	87.2	72.7	81.8	63.1	61.0	76	94.1
5-9 times	X <sub>8</sub> =2	21	86.2	67.0	73.3	58.3	54.8	19	93.6
≥10 times	X <sub>8</sub> =3	14	79.6	62.8	76.9	53.9	51.1	14	88.9
Total		116	86.3	70.6	79.7	60.9	59.2	109 <sup>2</sup>	93.9

Table IV Percentage mean of the ratios between observed and predicted values for different tasks of the expiratory function.

Quantification as described on page 41. The individual value is formed as a ratio between the observed, individual values and the predicted values multiplied by 100.

<sup>1</sup> No = 116, except for attack hours: No = 113 and sickness points 1957-61: No = 115

<sup>2</sup> N = 109 except for attack hours: No = 106 and sickness points 1957-61: No = 108

trials table (Table 22). In the first analysis (Table 21) the age at onset and duration are also shown as factors of some influence. In consideration of the fact that age, age at onset and duration show a high degree of mutual correlation (multicollinearity) however greater regard should be paid to the results given in Table 22 with respect to the coefficients for age and duration.

With regard to the variables  $FEV_0$  and  $FEV_{10}$  the duration and sickness points during the period 1957-61 are shown to be of some influence, which was only significant, however for  $FEV_{10}$  (see Table 21). The total sickness points appeared to be of little importance, at least as shown by the analysis in which the points for 1957-61 were included. When the points for this period were not included as a separate variable (see the second regression analysis, Table 22) the total sickness points then had higher values of coefficient. This may indicate that the correlation of the total sickness points to lung function reduction depended essentially on the intensity of the disease during the years almost immediately preceding the investigation. (The intensity of the disease = total number of sickness points during the period referred to divided by this period expressed in years.)

To elucidate this question further  $FEV_{10}$  was correlated to the intensity of the disease from its onset up to the end of 1956. This correlation was -0.10, i.e. weak, which suggested that the intensity of the disease during the period before 1957 was of only minor influence at least to this lung function variable. A corresponding calculation

between  $FEV_{10}$  and the intensity in 1957-61 showed a correlation of up to -0.33 which indicates that the intensity of the disease during the more recent years was significantly (\*) correlated to this lung function variable. The partial correlation between the disease intensity before 1957 and the lung function as measured by  $FEV_{10}$  at the present investigation—eliminating the influence of the disease in terms of 1957-61—was weak, i.e. 0.01.

An analogous calculation with regard to  $FEV_0$  showed that the partial correlation was of the same order of size.

With regard to the variables  $SVV_0$  and  $SVV_{10}$ , it was found in the first regression analysis that the sickness points during the period 1957-61 were of significant influence. Duration, age and number of attack hours also, however had some influence. The total sickness points, on the other hand, were found in the first analysis to be of no appreciable importance (Table 21).

In the second limited analysis (Table 22) the age factor was found to be significant, and some influence could also be ascribed to the total number of sickness points. The duration, on the other hand, was of less importance. In calculating the predicted values consideration was taken of the fact that in the control material also the test performance would have decreased with increasing age even in normal persons these two lung function variables in particular are strongly dependent on age.

The degree of correlation between

but only the following four factors were studied: sex, age, sickness points and duration. The results are given in Table 22. The motivation for this limited version was to obtain firstly a more general analysis comprising only the more "rough" factors, and secondly an analysis in which the age at onset was not included — since this age was practically identical with the actual age

of the patient minus duration similarly in years.

The analysis shows that as regards the first lung function variable VC, the factors found typically in the case histories of asthma patients were of no appreciable influence. Only the age factor appears to have any noteworthy effect — but not significant — and this is shown by the second regression ana-

Independent variable	Variable notation	Notation of regr. coeff.	Dependent variables							
			VC	FEV <sub>1.0</sub>	FEV <sub>2</sub>	MMV <sub>40</sub>	MMV <sub>1</sub>	TLC	FRC/TLC	RV/TLC
Sex	X <sub>1</sub>	b <sub>1</sub>	-1.9	0.1	0.4	-0.3	-1.0	1.1	-0.9	-0.7
Age	X <sub>2</sub>	b <sub>2</sub>	3.3	1.7	-1.7	4.0	5.0	1.5	0.3	3.9
Total sickness point	X <sub>3</sub>	b <sub>3</sub>	0.1	-1.2	0.5	0.7	0.4	1.1	4.2	12.5**
Duration	X <sub>4</sub>	b <sub>4</sub>	-3.2	-5.8	-7.8	-1.1	-5.7	3.9	2.5	5.3
Age at onset	X <sub>5</sub>	b <sub>5</sub>	-1.0	-3.0	-3.7	-3.8	-3.8	1.2	6	0
Attack hours	X <sub>6</sub>	b <sub>6</sub>	-0.6	-1.1	-2.0	-3.3	-3.2	-0.6	-0.1	-0.8
Sickness points 195-196	X <sub>7</sub>	b <sub>7</sub>	-1.5	-1.5	-1.5	-6.7	-6.7*	-1.0	1.2	2.3
Admission to hospital	X <sub>8</sub>	b <sub>8</sub>	-1.1	-0.8	-0.4	0.4	-0.6	-1.9	-0.2	-1.3

Table 21 Coefficients calculated in regression analyses comprising eight factors and — as dependent variable — lung function indices

Quantification as described on page 41

Independent variable	Variable notation	Notation of regr. coeff.	Dependent variables							
			VC	FEV <sub>1.0</sub>	FEV <sub>2</sub>	MMV <sub>40</sub>	MMV <sub>1</sub>	TLC	FRC/TLC	RV/TLC
Sex	X <sub>1</sub>	b <sub>1</sub>	-1.5	0.2	0.6	-0.1	0.8	-0.8	-10.0	-10.8
Age	X <sub>2</sub>	b <sub>2</sub>	3.3	3.5	0.3	6.0	7.1**	1.0	-3.8*	-2.1
Total sickness point	X <sub>3</sub>	b <sub>3</sub>	0	-3.5	-2.4	-3.8	-3.8	-0.5	3.7	0.7
Duration	X <sub>4</sub>	b <sub>4</sub>	-0.7	-3.8	-5.3	-1.2	-2.9	3.3	1.0	1.0

Table 22 Coefficients calculated in regression analysis comprising four of the eight factors included in Table 21 and — as dependent variable — different lung function indices

Quantification as described on page 41

ards the ratio between the residual volume and total lung capacity the total number of sickness points for the whole period after the onset of the asthma, and sex, appeared to be of noteworthy influence. The relationship between the total number of sickness points and the RV/TLC ratio did not thus appear to depend essentially on the intensity of the disease during the years almost immediately preceding the investigation, as seemed to be the case with the ventilation capacity

separately. As they were relatively small in number and no significant differences were shown between the different sex and age groups, the results are presented independently of these two factors.

In Table 23 these 12 patients are distributed into groups according to the estimated total number of sickness points (see page 25) for bronchial asthma since the onset of this disease. The majority of the patients are assigned to group C, i.e. they had at least 2000 points or more. The mean number of sickness points for these 12 patients is about 1, the rest of the asthma series is somewhat high (cf. Table 2).

Table 24 shows the mean values and ranges for the ventilation capacity and lung volumes, as percentages of the predicted values, for these 12 patients. If this table is compared with Table 19, it is seen that the mean values for the ratios for the different tests of ventilation capacity is lower for this group of patients than for the rest of the asthma series. With regard to the lung volumes and the mutual relationships between them, this group differs somewhat less on an average from the rest of the material.

#### Patients with a chronic disease in addition to the bronchial asthma

Of the 128 patients in the asthma series, 12 (8 men and 4 women) were diagnosed as having chronic disease in addition to the bronchial asthma. Since it was considered that this disease might influence the results of the analyses, these patients have been reported

Group according to sickness points	No.	Sickness points mean	Sickness points range
A ( <1000 p.)	2	464	432—495
B (1000—1999 p.)	3	1406	1316—1653
C ( ≥2000 p.)	7	2363	2034—2666
Total	12	2130	432—2666

Table 23. Distribution of groups according to estimated total number of sickness points for 12 patients who besides bronchial asthma had another chronic disease

	FEV <sub>1</sub> % of pred.	FEV <sub>2</sub> % of pred.	MVV <sub>10</sub> % of pred.	MVV <sub>15</sub> % of pred.
men range	43.9 19.1—70.0	67.8 41.7—106.8	35.0 23.3—69.4	23.8 20.2—40.1
	VC % of pred.	TLC % of pred.	FRC/TLC % of pred.	RV/TLC % of pred.
women range	80.0 37.8—81.8	81.9 62.5—116.1	131.2 113.2—145.1	167.7 123.8—237.7

Table 24. Mean values and ranges of ventilation capacity and lung volumes for 12 patients with chronic disease besides bronchial asthma, as percentages of the predicted values (prediction according to sex, age, height and weight)



the lung function variable  $MV\dot{V}_1$  and the estimated number of attack hours during the five years 1957-61 was studied. This was not found to be especially high; the correlation was  $-0.20$  (\*) but that between the number of attack hours before 1957 and the variable  $MV\dot{V}_1$  was only slightly less  $r = -0.15$ .

With regard to the variable TLC, the only factor of any influence — not, however significant — was the duration, and this is shown by both analyses (Tables 21 and 22).

With regard to the variables FRC/TLC and RV/TLC, both regression analyses showed that sex and the total sickness points had considerable influence. According to the first analysis the age at onset and duration were also of importance. Special attention should be paid to the fact that for both FRC/TLC and RV/TLC the total sickness points were shown to be of some influence (significant for RV/TLC) even in the analysis where the points for the period 1957-61 were regarded as an independent variable. This appears to indicate that the correlation of the sickness points with the variables FRC/TLC and RV/TLC — in contrast to that with  $FEV_{1.0}$ ,  $FEV_{1.5}$ ,  $MV\dot{V}_{40}$  and  $MV\dot{V}_1$  — depended essentially on the over all severity of the disease i.e. the total number of sickness points since the beginning of the bronchial asthma. In the case of the latter variables, on the other hand, the correlation of the total sickness points to the reduced lung function appeared to depend essentially on the intensity of the asthma during the years 1957-61.

To summarize these regression ana-

lyses indicate a considerable relationship between the reduction in ventilation capacity and the estimated severity of the disease during the years almost immediately preceding the investigation. If the sickness points for these later years were not regarded as an independent factor in the analysis, the total sickness points, i.e. the estimated over all degree of severity of the disease since its onset emerged as a factor of noteworthy influence (See Table 20 which shows the correlation between pairs of  $X$  and  $y$  variables). The correlation of the total sickness points with the lung function reduction thus appears to depend essentially on the degree of severity of the asthma during the years recently preceding the investigation.

With regard to the ventilation capacity — as estimated from the maximum voluntary ventilation i.e. the function variables  $MV\dot{V}_{40}$  and  $MV\dot{V}_1$  — age appeared to be an essential factor; this capacity decreasing with increasing age. Age which influenced the performance even of persons in the control material, appeared to have even greater weight as regards the asthma series.

This was also valid to a certain extent for the relationship between age and vital capacity. The value for the total lung capacity appeared to increase with the length of duration of the disease. These relationships were not significant. With regard to the ratio between the functional residual capacity and total lung capacity noteworthy influence could be ascribed to sex, age and possibly total number of sickness points and duration. As reg-

made, group C differed significantly ( ) from group A.

When the material was divided into three groups according to the duration of the asthma (Table 26) (group 1 duration of <15 years, group 2 15—24 years, group 3 ≥25 years) the observed mean value for the men in both group 1 and group 3 differed significantly (\*) and (\*\*) respectively) from the predicted value.

With regard to the women, all groups differed significantly from the predicted value (group 1 group 2 and group 3 \*)

Neither for the men nor for the women, nor when the sexes were com-

bined was there a significant mutual difference between the three groups or between combinations of the groups.

When the material was divided into three groups (Table 27) according to the age at onset (group 1 onset before or at the age of 16 years, group 2 onset after the age of 16 years but before the age of 40 years, group 3 onset at or after the age of 40 years) the observed mean value for the men in groups 2 and 3 differed significantly ( ) from the predicted value. The difference with regard to the men in group 1 was not significant. No significant difference was found between the different groups.

Groups according to estimated total % of values points		Work load, percentage of predicted value at											
		heart rate 130 beats/min						max. hr/min performed work					
		No. men	range	SD	SE	% of patients at steady state	No. men	range	SD	SE	% of patients at steady state		
<1000 p	M	11	83.8	38.5—130.8	31.3	9.4	82	11	97.6	45.6—123.5	25.5	7.7	53
1000—1999 p	M	16	71.1	36.9—121.5	23.6	6.7	75	16	86.1	41.0—118.1	30.2	5.1	81
≥2000 p	M	14	78.9	38.5—111.5	23.3	6.6	81	16	73.9	33.8—118.1	27.4	7.8	43
<1000 p	F	25	87.6	36.5—163.5	36.9	6.3	80	25	79.1	40.8—136.3	21.9	4.4	82
1000—1999 p	F	24	78.3	27.2—126.8	20.5	6.2	67	23	83.3	30.8—151.1	26.6	5.3	68
≥2000 p	F	21	82.3	42.6—121.7	23.4	4.9	62	23	69.7	0.0—131.9	23.3	5.9	57

Table 25  $\bar{W}_{130}$  and  $\bar{W}_{max}$  as percentage of predicted value, for the material classified in groups according to sex and estimated total number of attacks per 1 since the manifestation of the asthma.

Groups according to duration of the bronchial asthma		Work load, percentage of predicted value at											
		heart rate 130 beats/min					maximum performed work						
		sex	No. men	range	SD	SE	% of patients at steady state	No. women	range	SD	SE	% of patients at steady state	
<1 yr	M	11	71.7	37.5—121.5	27.8	8	11	60.8	33.0—123.5	20.1	6.1	82	
1—14 yr	M	17	86.3	38.9—111.5	22.3	7.3	85	17	80.5	41.8—123.3	28	7.0	71
≥15 yr	M	13	62	35.5—106.3	19.2	5.3	60	18	74.3	33.8—109.3	22.0	6.1	31
<13 yrs	F	20	86.7	30.5—163.5	20.4	6.6	45	26	78.8	40.8—136.3	23.2	5.2	55
13—21 yrs	F	23	76.1	20.9—121.5	28.8	5.1	69	21	73.8	0.0—151.1	26.8	4.0	59
≥22 yrs	F	18	79.5	43.5—120.5	23.1	5.9	81	19	65.7	40.8—136.1	27.4	6.3	63

Table 26  $\bar{W}_{130}$  and  $\bar{W}_{max}$  percentage of predicted value for the material classified into groups according to sex and duration of the bronchial asthma.

## DETERMINATION OF PHYSICAL WORK CAPACITY WITH SUBMAXIMAL WORK TEST

The physical work capacity was determined for all the 128 patients in the *asthma series*. The 12 patients who in addition to bronchial asthma had an other chronic disease of significance are reported separately (see page 71). Tables 31 and 32 (pages 72-75) show all the variables recorded or calculated in connection with the work test both for the entire material distributed according to sex and age, and also for the different groups according to the estimated total number of sickness points. Tables 29-30 show the work load given in per cent of predicted  $\text{Lpm/min}$  calculated from the observed data by interpolation or extrapolation (= rate of work) at heart rate 130 ( $W_{130}$ ) and also the maximum work performed at the work test ( $W_{\max}$ ) for the *asthma series* grouped according to different case history data e.g. duration, age at onset etc. Figs 2-4 show the relationship between the calculated physical work capacity at heart rate 130 with both the total hemoglobin content and the cardiac volume in the prone position on classification of the patients according to the estimated total number of sickness points. For the method used in the determination of circulatory functions reference may be made to page 27.

The results from both  $W_{130}$  and  $W_{\max}$  will be described separately below. In both cases comparison is made with the control material, and the influence of different case history data is discussed. Comparison is also made between the *asthma series* and the

control material with regard to the other variables recorded or calculated in connection with the work test.

### Work load at heart rate 130 ( $W_{130}$ )

The difference between the observed mean value expressed as a percentage of the predicted value, and this latter value (100 %) was significant for both men and women (\*\*\*) No significant difference was found between the three age groups.

On classification of the material into three groups (Tables 25-31-32) according to the estimated total number of sickness points (group A <1000 points, group B 1000-1099 points, group C  $\geq 2000$  points, see page 33) the mean value for the men in group A was somewhat lower than, but did not differ significantly from the predicted value, while the values for the men in groups B and C, on the other hand, were significantly lower than predicted (\*\*\*) Groups B and C had a lower mean value than group A but did not differ significantly either from group A or from each other, neither did the combined groups B and C differ significantly from group A.

With regard to the women each of the groups differed significantly from the predicted value (group A \*\* group B and group C \*\*\*). The groups did not differ significantly from one another and neither did the combined groups B and C differ significantly from group A.

When no sex classification was

into three groups (Table 29) according to the mean number of sickness points per year during the 3-year period 1957-61 (group 1 <60 points, group 2 60-99 points, group 3  $\geq 100$  points) the mean value for the men in all groups differed significantly from the predicted value (\* and \*\*\* for groups 1, 2 and 3 respectively)

With regard to the women, groups 1 and 3 differed significantly from the predicted value (\* and \*\* respectively)

Neither for the men nor for the women, nor when the sexes were combined was there a significant mutual difference between the three

groups or between combinations of the groups.

When the material was classified into two groups (Table 30) according to the number of admissions to hospital on account of bronchial asthma (group 1 1-4 admissions, group 2 5 or more admissions) the mean value of both the men in the two groups and the women in the two groups differed significantly (\*) from the predicted value.

There was no significant mutual difference between the groups for either sex. No significant difference was found between the two groups when the sex factor was disregarded.

Groups according to estimated % of sickness points for 1957-61	Sex	Work load, percentage of predicted value at										
		heart rate 120 beats/min					maximum performed work					
		%	mean	range	SD	SE	%	mean	range	SD	SE	
< 60 p.	M	21	84.8	39.3-141.6	27.3	5.8	83	29	57.0	41.0-125.3	22.8	5.1
60-99 p.	M	10	80.6	36.9-119.5	25.1	7.9	60	16	4.8	35.8-107.6	31.5	6.8
≥ 100 p.	M	8	65.2	28.5-96.9	20.2	7.3	75	8	68.9	38.0-109.5	24.5	8.7
< 60 p.	F	45	75.8	36.5-131.4	28.1	3.9	67	46	76.7	40.0-130.3	22.9	3.5
60-99 p.	F	16	87.4	25.5-165.8	36.5	9.1	62	17	82.2	26.8-161.1	26.2	6.3
≥ 100 p.	F	9	70.8	43.8-109.3	22.7	7.6	67	10	73.4	9.0-131.9	25.5	11.2

Table 29.  $W_{120}$  and  $W_{max}$  as percentage of predicted value for the material classified into groups according to the estimated average number of sickness points per year for the period 1957-61

Groups according to % of times admitted to hospital for bronchial asthma	Sex	Work load, percentage of predicted value at										
		heart rate 120 beats/min					max. work performed work					
		%	mean	range	SD	SE	%	mean	range	SD	SE	
1-4 times	M	37	83.9	38.5-141.6	26.0	8.0	83	27	84.5	41.8-125.5	23.0	4.4
$\geq 5$ times	M	14	74.9	36.0-121.5	27.5	7.3	87	14	75.1	35.5-118.1	27.8	7.7
1-4 times	F	42	78.2	29.2-163.6	31.1	4.4	67	52	77.5	9.0-151.1	27.8	2.7
$\geq 5$ times	F	21	74.9	36.5-116.8	22.3	6.9	52	21	77.7	40.0-102.8	24.1	6.3

Table 30.  $W_{120}$  and  $W_{max}$  as percentage of predicted value for the material classified into groups according to the number of times admitted to hospital for bronchial asthma

With regard to the women the observed mean value for all three groups differed significantly from the predicted value (group 1 \*\*\* group 2 \*\* group 3 \*) Groups 1 and 2 combined differed significantly (\*) from group 3

When no sex classification was made group 3 differed significantly (\*) from group 1 Otherwise the difference between the groups was not significant

When the material was classified into three groups (Table 28) according to the estimated number of attack hours (group 1 1-499 hours group 2 500-2499 hours group 3  $\geq 2500$

hours) the observed mean value for the men in group 1 did not differ significantly from the predicted value while the mean value for those in groups 2 and 3 differed significantly (\*\*\*)

With regard to the women, each of the groups differed significantly from the predicted value (\*\*\*)

There was no significant mutual difference between the three groups or between combinations of the groups either for the men or for the women. There was still no significant mutual difference between the groups when the sex factor was disregarded.

When the material was classified

Groups according to age at manifestation of bronchial asthma	Sex	Work load, percentage of predicted value at											
		heart rate 130 beats/min						maximum performed work					
		No	mean	range	SD	SE	% of patients at steady state	No	mean	range	SD	SE	% of patients at steady state
≤10 yrs.	M	11	88.8	60.0-141.5	23.9	7.8	83	11	8.1	33.8-123.3	20.1	9	36
17-30 yrs.	M	16	8.0	38.5-121.5	5.4	6.4	63	16	82.3	33.6-118.1	21.0	5.4	63
≥40 yrs.	M	14	7.1	36.0-121.5	28.7	7.7	79	14	82.7	41.0-125.5	28	7	79
≤10 yrs.	F	10	71.3	38.9-111.4	24.4	7.0	80	10	74.3	45.5-101.9	17.6	5.6	80
17-30 yrs.	F	33	72.3	29.3-170.6	27.1	4.7	66	23	73.6	9.0-186.1	23.4	4.3	54
≥40 yrs.	F	27	82.5	36.5-162.5	30.7	5.9	63	28	83.7	66.8-151.1	28	5.4	81

Table 27.  $\bar{V}_{130}$  and  $\bar{V}_{max}$  as percentage of predicted value for the material classified into groups according to sex and age at manifestation of bronchial asthma

Groups according to estimated total % of attack hours		Work load, percentage of predicted value at											
		heart rate 130 beats/min						maximum performed work					
		No	mean	range	SD	SE	% of patients at steady state	No	mean	range	SD	SE	% of patients at steady state
1-499 hrs.	M	12	90.8	38.5-130.6	27.3	7.0	83	12	92.9	38.7-133.5	22.4	6.8	87
500-2499 hrs.	M	10	73.6	44.0-118.5	22.6	7.1	90	10	77.9	41.0-109.5	20.8	6.5	79
≥2500 hrs.	M	17	6.3	36.0-141.5	28.9	6.6	69	17	72.6	33.8-118.1	26.0	6.3	47
1-499 hrs.	F	23	78.3	36.3-131.4	30.0	6.2	63	25	82.0	9.0-151.1	33.8	6.8	46
500-2499 hrs.	F	19	71.5	36.5-121.1	24.4	5.6	63	20	70.8	43.3-101.0	18.1	4.0	53
≥2500 hrs.	F	37	78.0	29.3-121.7	29.0	5.0	67	27	3.6	40.0-131.9	22.1	4.5	67

Table 28.  $\bar{V}_{130}$  and  $\bar{V}_{max}$  as percentages of predicted value for the material classified into groups according to sex and estimated total number of attack hours since the manifestation of the bronchial asthma

into three groups (Table 20) according to the mean number of sickness points per year during the 5-year period 1957-61 (group 1 <50 points, group 2 50-99 points, group 3  $\geq 100$  points) the mean value for the men in all groups differed significantly from the predicted value (\*) and for groups 1, 2 and 3 respectively).

With regard to the women, groups 1 and 3 differed significantly from the predicted value (\*\*\*) and \* respectively).

Neither for the men nor for the women, nor when the sexes were combined was there a significant mutual difference between the three

groups or between combinations of the groups.

When the material was classified into two groups (Table 30) according to the number of admissions to hospital on account of bronchial asthma (group 1 1-4 admissions, group 2 5 or more admissions) the mean value of both the men in the two groups and the women in the two groups differed significantly ( ) from the predicted value.

There was no significant mutual difference between the groups for either sex. No significant difference was found between the two groups when the sex factor was disregarded.

Groups according to estimated No. of sickness points for 1957-61		Work load, percentage of predicted value 1											
		heart rate 130 beats/min						maximum performed work					
		No.	mean	range	SD	SE	% of patients at steady state	No.	mean	range	SD	SE	% of patients at steady state
Sex													
< 50 p.	M	22	84.8	38.5-111.5	27.3	5.8	82	22	67.0	41.0-125.3	23.8	5.1	80
50-99 p.	M	18	80.6	36.9-110.5	25.1	7.0	80	10	4.5	35.8-102.0	21.3	6.8	70
≥ 100 p.	M	8	65.3	35.0-90.9	20.2	7.2	75	8	68.9	35.6-109.5	24.5	8.7	75
< 50 p.	F	48	73.8	36.8-131.4	26.1	3.0	83	48	76.7	40.0-136.3	23.9	3.5	82
50-99 p.	F	16	87.4	39.3-105.8	26.8	9.1	83	17	82.2	36.8-131.1	26.2	6.2	71
≥ 100 p.	F	9	76.8	43.8-102.3	22.7	7.0	87	10	73.4	40.0-131.9	25.5	11.2	76

Table 20.  $\bar{W}_{100}$  and  $\bar{W}_{max}$  as percentage of predicted value for the material classified in three groups according to estimated average number of sickness points per year for the period 1957-61.

Groups according to No. of times admitted to hospital (or bronchial) attacks	Sex	Work load, percentage of predicted value at											
		heart rate 130 beats/min						maximum performed work					
		No.	mean	range	SD	SE	% of patients at steady state	No.	mean	range	SD	SE	% of patients at steady state
1-4 times	M	37	83.9	36.5-111.5	26.0	5.0	83	27	81.5	41.0-125.5	23.0	4.4	70
$\geq 5$ times	M	14	71.9	36.9-121.5	27.5	7.3	87	14	72.1	35.8-118.1	27.8	7.7	43
1-4 times	F	49	78.3	30.3-105.3	31.1	4.4	67	53	77.5	40.0-151.1	27.0	3.7	60
$\geq 5$ times	F	31	71.9	30.6-116.8	23.3	4.0	82	21	77.7	40.0-102.0	24.1	5.3	67

Table 21.  $\bar{W}_{100}$  and  $\bar{W}_{max}$  as percentage of predicted value for the material classified in two groups according to extent and number of times admitted to hospital for bronchial asthma.

With regard to the women the observed mean value for all three groups differed significantly from the predicted value (group 1 \*\*\* group 2 \*\* group 3 \*) Groups 1 and 2 combined differed significantly (\*) from group 3

When no sex classification was made group 3 differed significantly (\*) from group 1 Otherwise the difference between the groups was not significant

When the material was classified into three groups (Table 28) according to the estimated number of attack hours (group 1 1-499 hours group 2 500-2499 hours, group 3  $\geq 2500$

hours) the observed mean value for the men in group 1 did not differ significantly from the predicted value, while the mean value for those in groups 2 and 3 differed significantly (\*\*\*)

With regard to the women each of the groups differed significantly from the predicted value (\*\*\*)

There was no significant mutual difference between the three groups or between combinations of the groups either for the men or for the women. There was still no significant mutual difference between the groups when the sex factor was disregarded

When the material was classified

Groups according to age at manifestation of bronchial asthma	Sex	Work load percentage of predicted value at											
		heart rate 130 beats/min						maximum performed work					
		No	mean	range	SD	SE	% of patients at steady state	No	mean	range	SD	SE	% of patients at steady state
≤10 yrs.	M	11	88.8	60.0-141.5	24.0	7.8	82	11	76.1	33.5-125.3	26.1	9	36
17-30 yrs.	M	18	8.0	38.5-121.5	23.4	6.4	83	16	82.3	88.6-116.1	21.6	5.4	83
≥40 yrs.	M	14	7.1	36.0-121.5	28.7	7.7	79	14	82.7	41.0-125.5	28		79
≤16 yrs.	F	10	71.3	36.9-114.4	21.4	7.9	80	10	74.3	45.5-101.0	1.8	5.6	80
17-39 yrs.	F	33	72.3	29.2-126.5	27.1	4.7	88	25	73.6	9.0-186.1	23.4	4.3	51
≥40 yrs.	F		85.5	36.5-163.5	30.7	5.9	68	28	82.7	36.8-161.1	28	5.4	64

Table 27.  $W_{50}$  and  $W_{max}$  as percentage of predicted value for the material classified into groups according to sex and age at manifestation of bronchial asthma

Groups according to estimated total No of attack hours		Work load, percentage of predicted value at											
		heart rate 130 beats/min						maximum performed work					
		No	mean	range	SD	SE	% of patients at steady state	No	mean	range	SD	SE	% of patients at steady state
1-499 hrs.	M	12	90.8	38.5-130.5	27.3	7.9	83	11	91.9	58.7-135.5	23.4	8.8	67
500-2499 hrs.	M	10	73.5	44.6-118.5	22.5	7.1	90	10	77.9	41.0-109.0	20.6	6.5	70
≥2500 hrs.	M	17	70.3	36.9-141.5	26.9	6.5	59	17	72.6	35.8-118.1	26.0	6.3	47
1-499 hrs.	F	23	78.3	36.3-131.4	30.0	6.2	65	23	82.9	9.0-151.1	33.8	6.8	56
500-2499 hrs.	F	19	77.8	36.3-124.1	24.4	5.6	83	20	76.8	43.3-101.0	18.1	4.0	83
≥2500 hrs.	F	27	72.0	39.2-121.7	26.0	5.0	67	27	73.6	40.0-131.9	23.1	4.5	63

Table 28.  $W_{50}$  and  $W_{max}$  as percentage of predicted value for the material classified into groups according to sex and a limited total number of attack hours since the manifestation of the bronchial asthma.

the men in groups 2 and 3 showed a significant difference ( ) Group 3 differed significantly ( ) from group 1. When groups 2 and 3 were combined, the difference between their weighted mean value and the value for group 1 was significant ( ).

With regard to the women, each of the groups differed significantly from the predicted value (group 1 - group 2 and group 3 \* ). There was no significant mutual difference between the groups, and neither did group 1 differ significantly from the combined groups 2 and 3.

When no sex classification was made, the difference between group 3 and group 1 was significant ( ). The difference between group 1 and the combined groups 2 and 3 was also significant ( ).

When the material was classified into three groups (Table 29) according to the mean number of sickness points per year during the 5-year period 1957-61 the mean value for the men in all three groups differed significantly from the predicted value (group 1 - group 3 \*\*). No significant mutual difference between the groups was found, but a significant difference ( ) was seen between group 1 and the combined groups 2 and 3.

With regard to the women, all three groups differed significantly from the predicted value (group 1 - group 2 - group 3 ). There was no significant difference between the groups.

When no sex classification was made no significant difference between the groups was found.

On classification of the material into two groups (Table 30) according to

the number of admissions to hospital on account of bronchial asthma, the mean value for both the men in the two groups ( \*) and the women in the two groups (\*\*\*) differed significantly from the predicted value. Neither for the men nor for the women was there any significant difference between the two groups.

No significant difference was found between the two groups when the sex factor was disregarded.

### Relationship between physical work capacity and lung function

The correlation between work capacity ( $\dot{V}_{I,0}$  and  $\dot{V}_{max}$ ) and different lung function variables was studied. For the variables of both work capacity and lung functions, each patient is represented by the quotient between the observed and predicted value.

The study showed that  $\dot{V}_{I,0}$  was highly significantly correlated with VC ( correlation coefficient,  $r=0.48$ ). Further significant relationships were found with  $FEV_{1,0}$ ,  $FEV_{1,0}/\dot{V}_{I,0}$ ,  $\dot{V}_{I,0}/\dot{V}_{max}$ ,  $\dot{V}_{I,0}/VC$  ( $r=0.30-0.33$ ) and with FRC/TLC (  $r=0.20$ ). No significant relationship was found between  $\dot{V}_{max}$  and  $RV/TLC$ .

A similar study was made with regard to  $\dot{V}_{max}$ . This showed that  $\dot{V}_{max}$  was significantly correlated with VC,  $FEV_{1,0}$ ,  $\dot{V}_{I,0}$  and  $\dot{V}_{I,0}/\dot{V}_{max}$  ( $r=0.28-0.31$ ). The correlation between  $\dot{V}_{max}$  and  $FEV_{1,0}$  was also significant (  $r=0.22$ ). No significant relationship was found between  $\dot{V}_{max}$  and FRC/TLC or  $RV/TLC$ .



## Maximum performed work load ( $W_{ms}$ )

The difference between the observed mean value expressed as a percentage of the predicted value and this latter value (100 %) was significant (\*\*\*) for both sexes. For the women there was a significant difference (\*) between the youngest and the oldest group the latter group having on an average a higher value. For both sexes combined the difference between the oldest and middle age groups was significant (\*) the former having on an average a higher value. No other significant difference was shown between the three age groups.

When the material was classified into three groups (Tables 25 31-32) according to the estimated *total number of sickness points* the mean value for the men in group A did not differ significantly from the predicted value while the mean value for the men in groups B and C showed a significant difference (\*\*\*) There was no significant mutual difference between the groups and neither was there any significant difference between group A and groups B and C combined.

With regard to the women each of the groups differed significantly from the predicted value (group A and group C \*\*\* group B \*\*) There was no significant mutual difference between the groups, and neither did group C differ significantly from groups A and B combined

On classification of the material regardless of sex, a significant difference (\*) was found between groups C and A The difference between groups B

and A on the other hand, was not significant. No significant difference was shown between group C and groups A and B combined.

When the material was classified into three groups (Table 26) according to the *duration* of the asthma the observed mean value for the men in groups 2 and 3 differed significantly from the predicted value (\*\* and \*\*\* respectively)

With regard to the women, the mean value for all groups differed significantly from the predicted value (group 1 and group 2 \*\*\* group 3 \*)

Neither for the men nor for the women, nor when the sexes were combined was there a significant mutual difference between the three groups or between combinations of the groups.

When the material was divided into three groups (Table 27) according to the *age at onset* the observed mean value for the men in groups 1 2 and 3 differed significantly (\*\* \*\* and \* respectively) from the predicted value.

For the women also the observed mean value for all three groups differed significantly from the predicted value (group 1 \*\*\* group 2 \*\*\* group 3 \*\*)

There was no significant mutual difference between the three groups or between combinations of the groups for either sex. There was still no significant difference between the groups when the sex factor was disregarded.

When the material was divided into three groups (Table 28) according to the estimated number of *attack hours* the observed mean value for the men in group 1 did not differ significantly from the predicted value, while that for

force maximal exertion (and neither was this the intention) but that a relatively high degree of exertion was attained.

On classification of the asthma series into groups according to sex, age and estimated total sickness points, no significant difference was found for the above variables between these groups (except for the highest heart rate attained, which was dependent on age). Neither was any significant difference found between the control material and the asthma series on comparison between the sex and age groups.

For  $\dot{V}_{O_2}$  a mean  $\dot{V}_{O_2}$  of 721 kpm/min ( $SD=163$  kpm/min,  $SE=81$  kpm/min) was obtained for the men in the asthma series, and of 637 kpm/min ( $SD=113$  kpm/min,  $SE=16$  kpm/min) for the women. For the men in the control material a mean figure of 861 kpm/min ( $SD=181$  kpm/min,  $SE=28$  kpm/min) was obtained, and for the women 817 kpm/min ( $SD=142$  kpm/min,  $SE=21$  kpm/min).

For  $\dot{V}_{O_2}$  a mean figure of 876 kpm/min ( $SD=186$  kpm/min,  $SE=27$  kpm/min) was obtained for the men in the asthma series, and of 816 kpm/min ( $SD=132$  kpm/min,  $SE=20$  kpm/min) for the women. For the men in the control material a mean figure of 1033 kpm/min ( $SD=208$  kpm/min,  $SE=24$  kpm/min) was obtained, and for the women 923

kpm/min ( $SD=140$  kpm/min,  $SE=27$  kpm/min).

#### Patients with a chronic disease in addition to bronchial asthma

The 12 patients (6 men and 6 women) who were diagnosed as having an additional chronic disease — which it was considered might have had decisive influence on the cardio-respiratory functions — are reported separately. Since these patients were relatively small in number and no significant differences were found between the sex and age groups among them, these factors are disregarded in the presentation of the results.

For the distribution of these patients into groups according to the estimated total number of sickness points for bronchial asthma since the onset of this disease, reference may be made to Table 23, page 62.

The mean value of  $\dot{V}_{O_2}$  for these patients, as percentage of the predicted value was 63.0 (range 23.1—81.5 %). The corresponding mean  $\dot{V}_{O_2}$  for  $\dot{V}_{O_{2max}}$  was 54.5 % (range 23.1—80.3 %). Calculating the predicted value regard was taken of sex and age. The calculations were based on the values for 10 out of the 12 patients.

The physical work capacity for this group of patients was on an average somewhat lower than for the rest of the asthma series, which is evident by comparison with the data given in Tables 21 and 22.

To summarize it may be said that both for  $W_{130}$  and  $W_{max}$  the observed mean value was less than the predicted value for all groups distributed according to sex, age and different case-history factors. For several of these groups the difference between the mean observed value and the predicted value was significant.

Some significant differences were also found between the mean observed values for the sub groups of certain case-history factor classifications. As a rule however these differences did not reach the significance level.

With regard to  $W_{130}$  significant differences were shown between certain sub groups classified according to the estimated total number of sickness points and according to age at onset.

With regard to  $W_{max}$  significant differences were shown between certain sub-groups classified according to the estimated total number of sickness points, estimated number of attack hours, estimated mean number of sickness points per year during the five-year period 1957-61 and age.

For both  $W_{130}$  and  $W_{max}$  a significant relationship was shown with both the vital capacity and the lung function variables measuring ventilation capacity.

Neither for  $W_{130}$  nor  $W_{max}$  was any relationship found with the ratio of residual volume to total lung capacity.

### Other function variables determined in connection with the submaximal work test

The results for some other variables studied on testing physical work capacity

(Tables 31 and 32) will be given briefly below. These variables are the heart rate reaction to the orthostatic test, the maximum heart rate and maximum respiratory frequency during the work test and the lactate concentration in the blood immediately after the end of the work test. The mean value for each of these variables is given both for the male and the female patients. Two comparisons were made for each of the variables, firstly between groups classified according to sex, age and estimated total number of sickness points, and secondly between the control material and the corresponding sex and age groups in the asthma series. For these comparisons it will only be stated whether any significant difference was found, either between the groups in the asthma series or between the control material and the corresponding asthma patients.

In the orthostatic test the mean increase in heart rate in the men of the asthma series was found to be 13 beats/min and in the women 14 beats/min. This is a normal finding.

The highest mean heart rate attained during the work test was 161 beats/min in the men and 150 beats/min in the women. This reduced with increasing age and vice versa.

The highest mean respiratory frequency attained during the work test was 31 breaths/min in the men and 30 breaths/min in the women. This is a normal finding.

The mean lactate concentration in the blood immediately after the end of the work test was 7.2 mE/l in the men and 6.9 mE/l in the women. This meant that the work test did not en-

percentage of predicted value at										
heart rate 170 beats/min			maximum perf. runed work					Heart rate (max.) beats/min	Resp rate (max.) breaths/min	Blood lact. mEq/l
No.	kpm/min	% of patients in steady state	No.	kpm/min	% of pred.	% of patients in steady state	No.			
3	1018	33	3	1100	100.6	33	3	179	28	8.2
3	925	80	6	783	85.2	67	6	151	31	7.8
1	820	100	2	750	102.9	50	2	165	28	7.9
6	918	50	11	863	93.6	53	11	160	22	7.7
	1270			1600	125.5			190	40	11.1
	840			800	85.8			126	22	4.0
	301			321	25.6			19	6	2.4
	123.1			97.6	7.7			5.6	1.8	0.7
5	912	80	5	900	82.1	80	5	173	28	7.4
1	820	100	2	600	66.9	50	2	158	28	8.1
9	812	100	9	823	81.9	80	9	160	31	7.8
11	851	91	10	796	80.1	81	10	162	30	7.7
	1170			960	118.1			178	36	12.2
	820			300	41.0			181	21	3.6
	146			211	29.3			13	4	2.8
	41.1			82.6	8.1			3.2	1.0	0.7
4	920	50	5	900	75.3	60	5	168	23	8.1
2	725	50	4	823	109.9	50	4	169	23	8.3
2	933	6	5	680	63.7	20	5	162	21	7.6
9	881	33	11	787	73.9	43	11	160	22	8.1
	1220			1200	118.1			185	40	14.2
	700			300	25.8			122	22	2.8
	158			279	27.4			22	6	2.7
	52.1			71.6	7.3			6.9	1.6	0.7
12	832	56	12	918	83.7	82	12	173	26	8.7
1	781	60	12	667	72.7	85	12	187	31	7.2
9	831	67	16	650	65.1	63	16	153	23	7.7
20	878	62	41	740	81.2	61	41	161	31	7.2
	1270			1600	125.5			190	40	14.2
	840			300	25.8			122	22	2.8
	188			268	25.6			13	5	2.7
	30.9			41.9	4.0			2.8	0.8	0.4

Groups acc. to sickness points and age (yrs.)	Charac- teristic	Work load, kpm/min						
		Heart rate, beats/min			heart rate 130 beats/min			heart rate 150 beats/min
		No	rest	standing	No	kpm/ min	% of pred.	% of patients at steady state
A	30-49 mean	3	6	81	3	607	93.4	100
	50-59 "	0	69	83	6	632	97.2	83
	60-68 "	2	73	99	2	520	80.0	50
	Total A mean	11	69	84	11	605	93.0	82
	" highest		80	120		850	130.8	1000
	" lowest		50	54		250	38.5	420
	" SD		10.6	18.6		212	31.3	244
B	30-49 mean	5	70	84	5	538	80.8	80
	50-59 "	2	73	110	2	450	69.2	50
	60-68 "	9	6	88	9	468	0.5	8
	Total B mean	16	6	88	16	482	74.1	75
	" highest		104	116		790	121.5	970
	" lowest		53	62		240	36.9	430
	" SD		15.5	15.0		147	22.6	153
C	30-49 mean	5	60	79	5	582	89.5	80
	50-59 "	4	83	96	4	400	61.5	75
	60-68 "	5	60	83	5	531	82.2	40
	Total C mean	14	72	86	14	513	78.9	64
	" highest		92	110		920	141.5	1070
	" lowest		48	62		230	36.9	430
	" SD		12.8	12.6		164	25.3	156
Total A+B+C	30-49 mean	13	71	81	13	571	87.9	85
	50-59 "	12	74	90	12	524	80.6	75
	60-68 "	16	73	88	16	490	76.4	63
	Total mean	41	78	86	41	526	80.8	74
	" highest		104	120		920	141.5	1070
	" lowest		48	54		240	36.9	430
	" SD		13.5	14.4		174	26.5	183
	SE		2.1	2.3		27.2	4.1	30.9

Tabl 31 Observati ns connected with the submaximal work test in mal patients

range of predicted value ±										
heart rate 178 beats/min			maximum performed work				No.	Heart rate (max.) beats/min	Resp rate (max.) breaths/min	Blood lact. mg/L
lpm/min	of patients in steady state	No.	lpm/min	of pred.	% of patients in steady state					
1	1013	23	3	1109	100.0	23	3	178	26	8.3
1	925	80	6	783	85.2	87	6	181	31	7.5
1	829	100	3	750	107.8	60	3	183	28	7.9
1	918	50	11	883	97.6	53	11	189	27	7.7
	1970			1300	123.6			190	40	11.1
	840			800	83.8			126	22	4.0
	301			321	25.5			19	6	2.4
	123.1			97.6	7.7			8.6	1.8	0.7
8	943	80	3	900	82.1	80	8	178	26	7.4
1	620	100	2	600	66.0	50	2	168	26	8.1
3	813	100	9	632	81.9	80	9	186	31	7.8
11	834	91	16	706	80.1	81	16	183	30	7.7
	1170			908	118.1			178	26	12.3
	920			300	41.8			134	21	3.8
	146			211	20.3			13	4	2.6
	44.1			82.8	8.1			3.3	1.0	0.7
4	920	50	5	900	73.2	80	5	188	28	8.1
2	725	50	4	673	56.9	60	4	180	27	6.8
3	939	0	6	880	83.7	70	6	187	31	7.5
9	881	23	14	707	78.9	43	14	188	32	6.4
	1288			1300	118.1			185	40	14.2
	799			800	25.6			122	22	2.3
	168			379	27.4			22	6	2.7
	53.1			74.8	7.8			8.9	1.6	0.7
12	932	80	13	946	83.7	62	13	178	28	6.7
3	784	80	12	687	72.7	86	12	186	31	7.3
9	831	87	18	660	83.1	62	18	153	33	7.7
25	878	63	41	740	81.3	61	41	181	31	7.3
	1970			1500	123.6			190	40	14.3
	340			300	33.8			122	22	2.3
	168			388	25.6			18	3	2.7
	20.9			41.9	4.0			2.8	0.8	0.4

Groups acc. to sickness points and age (yrs)	Characteristic	Heart rate beats/min		Work load,						
				heart rate 130 beats/min			heart rate 150 beats/min			
				No	rest	standing	No	kpm/ min	% of pred.	% of patients at steady state
A	30-49 mean	12	79	94	12	323	78.6	67	12	438
	50-59	6	78	90	6	308	89.5	60	5	494
	60-68	8	6	94	8	340	84.3	50	5	384
	Total A mean	25	78	93	25	339	83.6	60	22	439
	" highest		98	118		680	165.3			660
	" lowest		54	72		150	36.5			230
	" SD		10.7	13.8		127	30.9			121
	" SE		2.1	2.8		25.4	6.3			25.1
B	30-49 mean	11	77	90	11	326	79.3	64	11	465
	50-59	7	80	101	6	232	61.3	83	5	402
	60-68	7	81	91	7	377	91.7	57	6	493
	Total B mean	25	79	93	24	322	8.5	67	23	456
	" highest		110	134		570	126.5			660
	" lowest		58	66		170	39.2			270
	" SD		15.5	18.1		125	30.5			116
	" SE		3.0	3.0		25.6	6.2			24.2
C	30-49 mean	6	83	96	6	268	65.2	67	6	393
	50-59	5	81	93	4	200	65.7	50	3	420
	60-68	12	79	92	11	301	73.2	64	6	406
	Total C mean	23	81	93	21	286	69.5	62	15	404
	" highest		104	115		500	121.7			560
	" lowest		61	72		180	43.8			260
	" SD		12.0	10.1		94	22.4			95
	" SE		2.6	2.2		20.6	4.9			24.5
Total A+B+C										
	30-49 mean	29	79	93	29	313	6.3	66	29	430
	50-59	17	80	95	18	206	72.0	67	14	439
	60-68	27	79	92	26	335	81.5	58	17	430
	30-68 mean	73	79	93	70	318	77.3	63	60	437
	" highest		110	134		680	165.5			660
	" lowest		54	66		120	39.3			230
	" SD		12.9	14.5		118	28.6			113
	" SE		1.6	1.7		14.2	3.4			14.6

Table 32 Observation connected with the maximal work test in female patients.

percentage of predicted value †							Heart rate (max.) beats/ min	Resp rate (max.) breaths/ min	Blood fact. ml/l	
heart rate 170 beats/min			maximum perf mixed work							
No.	kpm/ min	of patients † steady state	No.	kpm/ min	% of pred.	of patient † steady state				
9	557	56	12	830	72.5	50	12	174	28	6.9
2	848	50	5	480	87.7	60	9	154	23	8.8
3	457	8	8	330	83.6	80	8	141	30	8.2
16	258	43	25	472	79.1	52	23	180	30	6.1
	800			800	128.2			190	40	9.7
	450			200	40.0			116	22	2.1
	121			181	21.9			20	8	2.8
	22.9			30.3	4.4			40	0.9	0.5
9	584	56	11	845	74.9	61	11	167	25	7.2
4	520	73	7	407	78.4	85	7	149	29	7.8
2	615	50	7	429	104.6	81	7	148	31	7.9
15	671	60	23	474	83.2	68	23	157	29	7.2
	780			800	181.1			180	40	12.6
	380			200	30.6			101	20	2.1
	116			123	28.0			18	8	2.4
	30.8			28.6	8.3			2.6	1.8	0.5
6	517	22	6	600	68.5	22	6	172	29	8.1
2	640	0	3	290	82.6	40	6	142	28	7.6
3	320	87	12	323	77.8	76	12	142	32	8.6
11	522	30	23	367	69.7	57	23	156	29	7.1
	50			600	121.9			182	40	11.4
	218			80	9.0			98	18	2.7
	140			154	28.8			23	6	2.2
	42.4			27.2	8.9			8.2	1.2	0.8
21	587	50	20	628	72.4	52	20	171	28	7.2
8	555	50	17	294	72.2	65	17	148	31	7.1
8	524	28	27	363	86.2	62	27	125	31	8.2
44	560	48	18	446	77.8	60	73	186	30	8.9
	880			800	181.8			190	40	12.6
	310			80	9.0			98	18	2.7
	122			132	26.0			22	8	2.2
	19.6			17.9	3.0			2.6	0.8	0.2



## HEMODYNAMIC STUDIES

Right heart catheterization was performed without complications on 69 out of a total of 78 patients who had not reached the age of 60 years.

The remaining nine patients were unable to go into hospital for the catheterization, for various private reasons (e.g. a young mother with small children, an employer who was not able to leave his firm). These patients did take part, however, in the tests of lung function and physical work capacity that could be carried out in the outpatients department, and some idea of their cardio-respiratory function was still therefore obtained.

The distribution of the nine patients into groups according to their sickness points (see page 25) is shown in Table 33. This table also shows the corresponding distribution of all the 72 patients who had not reached the age of 60 years. (The six patients with another chronic disease besides the bronchial asthma, which it was considered might have had

decisive influence on the different functions, were excluded.)

The table shows that in this group of nine patients the estimated degree of severity of the asthma — as judged by the total number of sickness points — had been relatively mild. None of the patients were assigned to group C (see page 35) and no fewer than seven of the nine were assigned to group A. The mean number of sickness points for these patients was 681 while that for the entire material was 1587.

Table 34 shows the mean values and ranges for ventilation capacity, lung volumes and physical work capacity as a percentage of the predicted value. Tests of physical work capacity, ventilation capacity and vital capacity were performed on all nine patients, while investigations of the other lung volumes were performed on five patients. For comparison, the mean values for the material as a whole are given in parentheses in the table; these values are taken from Table 19, page 58.

Groups according to sickness points	No.	Sickness points mean
A ( <1000 p.)	7 (27)	488
B (1000—1999 p.)	2 (26)	1360
C ( ≥2000 p.)	3 (19)	—
Total	9 (72)	684 (1387)

Table 33 Distribution into groups according to estimated number of sickness points, for 9 patients below the age of 60 years who did not undergo heart catheterization. The corresponding figure for all of the 72 patients in the material who had not reached the age of 60 years, is given in parentheses.

	VC % of pred.	FEV <sub>1.0</sub> l / pred.	FEV <sub>0.5</sub> % of pred.	MM <sub>0.5</sub> % of pred.	MM <sub>1.0</sub> l / pred.
mean	93.7 (89.8)	86.9 (72.0)	91.8 (79.5)	79.4 (84.1)	80.1 (63.2)
range	88.2—103.0	75.9—100.0	81.7—101.5	70.0—86.3	70.0—102.0
	TLC l / pred.	FRC/TLC % of pred.	RV/TLC % of pred.	V <sub>120</sub> l / pred.	V <sub>max</sub> l / pred.
mean	83.1 (93.8)	97.0 (115.1)	113.0 (115.1)	83.0 (78.1)	83.2 (4.6)
range	71.4—92.9	78.7—110.8	89.0—131.2	36.5—114.4	60.6—136.3

Table 34 Ventilation capacity, lung volumes and physical work capacity as a percentage of the predicted value for patients who did not undergo heart catheterization. The corresponding mean figure for the whole group of patients who had not reached the age of 60 years is given in parentheses.

The tables concerning both the degree of severity of the asthma and the lung function and physical work capacity show that these nine patients, regarded as a group differed from the rest of the material in that their values for the above variables were on an average more favourable with the exception of the mean observed value for the total lung capacity which was somewhat lower than the corresponding value for the rest of the series.

Of the 60 patients who underwent heart catheterization, six had another chronic disease apart from the bronchial asthma (see page 160) which it was considered might have had a decisive influence on the cardio-respiratory functions. These patients were therefore not included in the analyses of the data obtained in the hemodynamic studies, but are reported separately (page 88, Table 38). The remaining 53 patients, 25 men and 28 women, thus constituted the material on which the analyses were based.

Since, however, for one of the men and one of the women values of 200 and 100 bpm/min respectively were obtained at the first load in the work test compared with 300 and 200 for the 116 men and women, these two patients were excluded from the work test analyses. They are however represented in the Figures showing the normal relationships of the tables. For neither of these patients were the hemodynamic values found to differ to any notable extent from the mean value for the group with regard to any of the different anoxic studies in the hemodynamic investigations.

## Results

The observations made during the heart catheterization, both during an ordinary resting state and during anoxic

anoxia and the graded work test, are given in Tables 37—44 pages 90—97.

The right atrial pressure was found to be normal in all patients during a resting state (Tables 37—38).

The mean value for the systolic pressure at rest (Tables 37—38) in the pulmonary artery was normal (normal values as given by Holmgren, Jonsson and Sjöstrand, 1900) i.e. 19 mm Hg both for the men and the women. The highest pressure recorded was 31 mm Hg (Table 37). During the graded work load test (Tables 41—44) an almost doubled systolic pressure was on an average observed. During standardized anoxic anoxia (Tables 39—40) the pressure in the men rose, on an average, to 31 mm Hg from 19 mm Hg at a normal resting state. The corresponding values for the women were 27 and 19 mm Hg respectively. For both sexes the increase during anoxic anoxia was significant (\*\*\*)

When the patients were classified into groups according to the estimated total number of sickness points, no noteworthy difference with regard to the systolic pressure was found between these groups either during a normal resting state, work load or anoxic anoxia.

The average value for the mean pressure at rest in the pulmonary artery was normal, i.e. 12 mm Hg for both men and women. The highest value measured was 20 mm Hg and this also lay within the normal limits. During the work load test an almost doubled mean pressure was on an average observed.

During anoxic anoxia the mean

pressure in the men rose on an average to 10 mm Hg from 12 mm Hg at a normal resting state. The corresponding values for the women were 17 and 12 mm Hg respectively. The increase during anoxic anoxia was significant for both sexes (\*\*\*) When the patients were classified into groups according to the estimated total number of sickness points a mean pressure of 30 mm Hg was obtained for the four men of ages 50—59 years and who had more than 2000 points, while the corresponding pressure for the eight men in the same age group who had not attained 2000 points was 19.5 mm Hg. This difference might be due to the fact that two of the four men who had attained more than 2000 points had about 7700 and 3300 points and a disease duration of 50 and 97 years respectively. Their mean pressures in the pulmonary artery were 47 and 44 mm Hg respectively. The other two men had only a few more than 2000 sickness points their pressures lay even during anoxic anoxia within the normal variation for the mean pressure in the pulmonary artery during a normal resting state.

No other noteworthy differences were found on classification of the patients into groups according to sex, age and sickness points, either during a normal resting state, work load or anoxic anoxia. No significant pressure gradients over the pulmonary valve were demonstrated.

The mean value for the diastolic pressure at rest in the pulmonary artery was 11.1 e. 7 mm Hg for the men and 11 mm for the women.

The pulmonary capillary venous

(PCV) mean pressure at rest lay within the normal variation in all patients. The highest pressure recorded was 11 mm Hg. In one patient a pressure of 15 mm Hg was observed during exercise. Disregarding this patient the highest observed value during work was 13 mm Hg. During exercise the mean value for the mean PCV pressures increased somewhat, but the difference on comparison with the corresponding value for the PCV pressure during a resting state was not significant.

The pulmonary vascular resistance (R) expressed as mm Hg per l/min

$\frac{\bar{P}_{PA} - \bar{P}_{PCV}}{Q}$  was determined at rest and

during exercise (Tables 37—38 41—44 and Figure 7). The pulmonary vascular resistance index ( $R_i$ ) expressed as mm Hg per l/min m<sup>2</sup> BSA,

$\frac{\bar{P}_{PA} - \bar{P}_{PCV}}{Q} \text{ BSA}$ , was also calculated

for the same patients. The mean pulmonary vascular resistance at rest for the men was 1.40 and for the women 1.41. On classification of the material into groups according to the estimated total number of sickness points no significant difference was found between these groups. Neither was there any significant difference between the pulmonary vascular resistance at rest and that during work.

The average systolic brachial pressure during a state of rest was 135 mm Hg for the men of ages 30—49 years and 151 mm Hg for those of ages 50—59 years. The corresponding values for the women were 130 and

138 mm Hg. The highest value observed among the men was 1.0 and among the women 205 mm Hg.

The average mean and diastolic brachial pressure at rest for the men of ages 30–49 years were 100 and 76 mm Hg respectively and for the men of ages 50–59 years 112 and 83 mm Hg respectively. The corresponding values for the women were 101 and 78 and 114 and 80 mm Hg, respectively.

In no subject were abnormally high or low arterial blood pressure changes observed during exercise.

On an average the oxygen uptake during heart catheterization was not significantly higher (3.2 %) than under BMR conditions, and was on an average 6.8 % higher than the predicted basal value at rest. (Predicted from sex, age, body height and body weight according to Harris and Benedict, 1910). During anoxia the oxygen uptake was on an average 9 % lower than during normal resting conditions in connection with the catheterization.

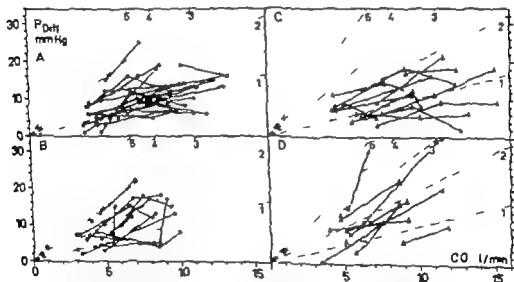


Fig 7 The relationship between pressure gradient ( $P_{Diff}$ ) over pulmonary vascular bed — pressed on the difference between pulmonary arterial mean pressure and pulmonary capillary venous mean pressure (in mm Hg) — and cardiac output (CO), l/min.

The interrupted lines represent different pulmonary vascular resistances (R). Circles represent females, triangles males. Open symbol represent the condition during rest, symbols with dot represent the condition during work, 300 kpm/min for females and 400 kpm/min for males. Filled symbols represent the condition during work, 400 kpm/min for females and 600 kpm/min for males.

Fig 7 A shows the conditions for females 30–49 years old, Fig 7 B for females 50–59 years old, Fig 7 C shows the conditions for males 30–49 years old, Fig 7 D for males 50–59 years old. For one symbol there was no room in Fig 7 D. This symbol (see arrow in the figure) represents  $R = 0.2$ ,  $P_{Diff} = 21$  mm Hg,  $CO = 2.6$  l/min.

pressure in the men rose on an average to 10 mm Hg from 12 mm Hg at a normal resting state. The corresponding values for the women were 17 and 12 mm Hg respectively. The increase during anoxic anoxia was significant for both sexes (\*\*\*) When the patients were classified into groups according to the estimated total number of sickness points a mean pressure of 30 mm Hg was obtained for the four men of ages 50—59 years and who had more than 2000 points, while the corresponding pressure for the eight men in the same age group who had not attained 2000 points, was 19.5 mm Hg. This difference might be due to the fact that two of the four men who had attained more than 2000 points had about 7700 and 3300 points and a disease duration of 50 and 3 years respectively. Their mean pressures in the pulmonary artery were 47 and 44 mm Hg respectively. The other two men had only a few more than 2000 sickness points their pressures lay even during anoxic anoxia within the normal variation for the mean pressure in the pulmonary artery during a normal resting state.

No other noteworthy differences were found on classification of the patients into groups according to sex, age and sickness points either during a normal resting state, work load or anoxic anoxia. No significant pressure gradients over the pulmonary valve were demonstrated.

The mean value for the diastolic pressure at rest in the pulmonary artery was normal i.e. 7 mm Hg for the men and 8 mm for the women.

The pulmonary capillary venous

(PCV) mean pressure at rest lay within the normal variation in all patients. The highest pressure recorded was 11 mm Hg. In one patient a pressure of 16 mm Hg was observed during exercise. Disregarding this patient the highest observed value during work was 13 mm Hg. During exercise the mean value for the mean PCV pressures increased somewhat but the difference on comparison with the corresponding value for the PCV pressure during a resting state was not significant.

The pulmonary vascular resistance (R) expressed as mm Hg per l/min

$$\frac{\bar{P}_{PA} - \bar{P}_{PCV}}{Q}$$

was determined at rest and during exercise (Tables 37—38, 41—44 and Figure 7). The pulmonary vascular resistance index ( $R_i$ ) expressed as mm Hg per l/min m<sup>2</sup> BSA

$$\frac{\bar{P}_{PA} - \bar{P}_{PCV}}{Q} \text{ BSA, was also calculated}$$

for the same patients. The mean pulmonary vascular resistance at rest for the men was 1.49 and for the women 1.41. On classification of the material into groups according to the estimated total number of sickness points no significant difference was found between these groups. Neither was there any significant difference between the pulmonary vascular resistance at rest and that during work.

The average systolic brachial pressure during a state of rest was 135 mm Hg for the men of ages 30—49 years and 161 mm Hg for those of ages 50—59 years. The corresponding values for the women were 130 and

and difference was found between different groups of the material on classification according to sickness points.

The mean oxygen saturation of mixed venous blood (drawn from the pulmonary artery) was 71.2 % (range 60.5—81.4) at rest. When the material was divided into groups according to sex, age and estimated total number of sickness points, no significant difference between these groups was found. During exercise the saturation decreased with increasing working intensity and heart rate (Figure 8). The lowest value observed was 24.0 %.

The mean arterio-venous oxygen difference (A<sub>VD</sub> ml/l) was 45.2 ml/l (range 29.7—59.7) at rest. When the material was divided into groups according to sex and estimated total number of sickness points no significant difference between these groups was found.

During anoxic anoxia the arterio-venous oxygen difference decreased by an average of 3.3 ml/l to an average

of 41.9 ml/l (range 27.7—64.2). When the material was divided into groups according to sex and sickness points, no significant difference between the different groups was shown for this value during anoxic anoxia.

During muscular work the A<sub>VD</sub> values increased with increasing working intensity. The highest values observed for the men were 111.2 ml/l at 300 kpm/min and 153.4 ml at 600 kpm/min. The corresponding figures for the women were 104.2 ml/l and 118.9 ml/l at 200 and 400 kpm/min respectively. In Figure 9 A<sub>VD</sub> is plotted against oxygen uptake, and in Figure 10 against heart rate at rest and during exercise.

Both for the resting state, anoxic anoxia and for the graded work test, the mean value for the A<sub>VD</sub> in the older age group was somewhat higher than in the younger age group.

The mean cardiac output at rest was 5.6 l/min for the men of ages 30—49 years and 4.8 for the men of ages 50—59 years. The corresponding cardiac indices were 3.0 and 2.7 respectively. For the women of ages 30—49 years the mean minute volume was 8.1 litres and for those of ages 50—59 years 4.2 litres. The corresponding cardiac indices were 3.0 and 2.5 respectively. On classification of the material into groups according to the estimated total number of sickness points, no noteworthy difference was observed between the groups.

During anoxic anoxia the cardiac minute volume increased for the men — in comparison with that during a resting state — by an average of 0.8 litre while for the women there was

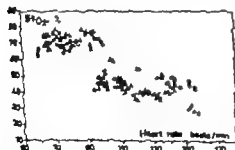


Fig. 8 Oxygen saturation of blood in the pulmonary artery (% ) per cent, in rest and heart rate (beats/min) at rest and during work.

Circles represent females and triangles males. Open symbols represent patients 30—49 years old, filled symbols patients 50—59 years old.

During exercise the oxygen uptake increased with increasing work intensity. The mean mechanical efficiency values were 31.0 % and 25.6 % for the men at 300 kpm/min and at 600 kpm/min respectively. The corresponding values for the women at 200 and 400 kpm/min were 19.7 % and 23.1 % respectively. For the entire material regardless of sex and graded load the efficiency value was 22.6.

A comparison between the rate of work and heart rate in the sitting position without cardiac catheterization and in the supine position with cardiac catheterization was made.

The difference between the working capacity in the sitting and supine positions was determined for both the heart rates 130 and 150. In 54 patients, regardless of sex, the  $W_{130}$  value in the supine position was 403 kpm/min i.e. 16 kpm/min higher on an average than that in the sitting position. At the higher heart rate level the  $W_{150}$  value in the supine position was 400 kpm/min i.e. 13 kpm/min higher on an average than that in the sitting position (30 patients). Neither for  $W_{130}$  nor  $W_{150}$  was the difference between the sitting and supine position significant.

The difference in heart rate between the sitting and supine positions at a certain given load was studied. A mean heart rate of 106 beats/min was recorded for 29 men during exercise at 300 kpm/min in the sitting position and 107 beats/min during similar exercise in the supine position. The corresponding value for 19 of the men during exercise at 600 kpm/min was 135 beats/min both in the sitting and supine positions. For 33 women a mean heart rate of 116 beats/min was recorded during exercise of 200 kpm/min in the sitting position and 113 beats/

min during similar exercise in the supine position. The corresponding values for 20 of the women during exercise of 400 kpm/min were 136 and 141 beats/min, respectively. Neither for the men nor for the women was the difference in heart rate between the sitting and supine positions at different work loads of a noteworthy order of size.

The mean arterial oxygen saturation at rest was 96.4 % (range 87.0—100.0). When the material was divided into groups according to sex, age and estimated total number of sickness points, no significant difference between the different groups was found, with regard to this value.

During anoxic anoxia the arterial oxygen saturation decreased for both sexes by an average of 20 per cent units to an average of 76.3 % (range 53.4—93.1). On classification of the material into groups according to age and sickness points, no significant difference between these groups was found for this value during anoxic anoxia.

During exercise of 300 kpm/min for men and 200 kpm/min for women the mean arterial oxygen saturation decreased somewhat, i.e. from an average of 96.0 % during a resting state to 95.0 %, and from 96.7 % to 96.1 % respectively. These differences were not however significant. Neither was there any significant difference for the men and women who performed the second work load test, i.e. at 600 and 400 kpm/min respectively, on comparison with the respective mean values for the arterial oxygen saturation during a resting state. No significant

ant difference was found between different groups of the material on classification according to sickness points.

The mean oxygen saturation of mixed venous blood (drawn from the pulmonary artery) was 71.3 % (range 60.6—81.4) at rest. When the material was divided into groups according to sex, age and estimated total number of sickness points, no significant difference between these groups was found. During exercise the saturation decreased with increasing working intensity and heart rate (Figure 8). The lowest value observed was 24 %.

The mean arterio-venous oxygen difference (AVD ml/l) was 45.2 ml/l (range 29—59.7) at rest. When the material was divided into groups according to sex and estimated total number of sickness points no significant difference between these groups was found.

During anoxic anoxia the arterio-venous oxygen difference decreased by an average of 3.3 ml/l to an average

of 41.9 ml/l (range 27.7—64.2). When the material was divided into groups according to sex and sickness points, no significant difference between the different groups was shown for this value during anoxic anoxia.

During muscular work the AVD values increased with increasing working intensity. The highest values observed for the men were 111.2 ml/l at 300 kpm/min and 133.4 ml at 600 kpm/min. The corresponding figures for the women were 104.2 ml/l and 118.9 ml/l at 200 and 400 kpm/min respectively. In Figure 9 AVD is plotted against oxygen uptake, and in Figure 10 against heart rate at rest and during exercise.

Both for the resting state, anoxic anoxia and for the graded work test, the mean value for the AVD in the older age group was somewhat higher than in the younger age group.

The mean cardiac output at rest was 5.0 l/min for the men of ages 30—49 years and 4.8 for the men of ages 50—69 years. The corresponding cardiac indices were 3.0 and 2.7 respectively. For the women of ages 30—49 years the mean minute volume was 5.1 litres and for those of ages 50—69 years 4.2 litres. The corresponding cardiac indices were 3.0 and 2.3 respectively. On classification of the material into groups according to the estimated total number of sickness points, no noteworthy difference was observed between the groups.

During anoxic anoxia the cardiac minute volume increased for the men — in comparison with that during a resting state — by an average of 0.3 litre, while for the women there was

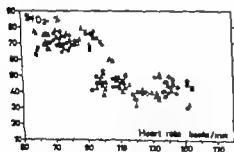


Fig. 8. Oxygen saturation of blood in the pulmonary artery ( $Sp_2$ ), per cent in relation to heart rate (beats/min) at rest and during work.

Circles represent females and triangles males. Open symbols represent patients 30—49 years old, filled symbols patients 50—69 years old.



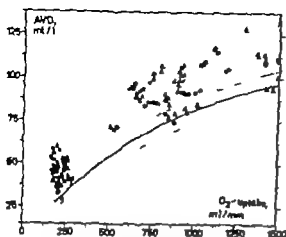


Fig 9. Arterio-venous oxygen difference (AVD) ml/l in relation to oxygen uptake ml/min STPD at rest and during work.

Central line indicates regression between these parameters in a control material. Interrupted lines indicate  $\pm$  standard error of estimate. The control material has been collected by Holmgren (personal communication) from results published by Holmgren et al. (1957, 1960) and Bevegård et al. (1960).

Symbols as in Fig 8.

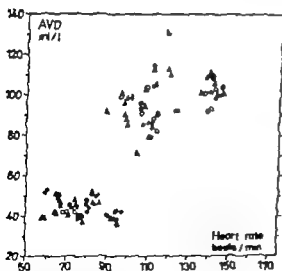


Fig 10. Arterio-venous oxygen difference (AVD) ml/l in relation to heart rate beats/min, at rest and during work.

Symbols as in Fig 8.

an average decrease of 0.4 litre. For neither sex was the difference significant. When the material was divided into groups according to the sickness points, no significant difference between these groups was found.

During exercise at 300 kpm/min for the men and 200 kpm/min for the women, the cardiac minute volumes were on an average 9.2 and 8.0 litres respectively. For those men and women who performed the second work load test i.e. 600 kpm/min and 400 kpm/min respectively, the cardiac minute volumes increased on an average to 12.7 and 10.1 litres. The relation of the cardiac minute volume to the oxygen uptake at rest and during exercise is shown in Figure 11. On classification of the material into sickness point groups no significant difference with regard to the minute volume was shown between these groups.

The mean stroke volume at rest was 72 ml for the men and 81 ml for the women. On classification of the material into sickness point groups no noteworthy difference was found between these groups.

The stroke volume during anoxic anoxia decreased in comparison with that during a resting state by an average of 8 ml for the men and 10 ml for the women. For neither sex however was the difference significant. Neither was there any significant difference when the material was divided into groups according to the number of sickness points.

In the men during exercise at 300 kpm/min and in the women during exercise at 200 kpm/min, the stroke volume increased — in comparison

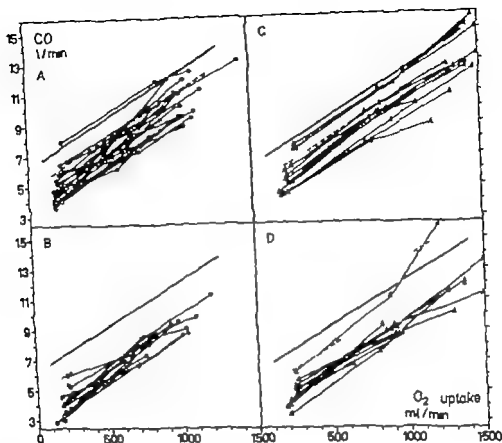


Fig. 11 Oxygen peak ml/min, STPD  $\dot{V}$  relation to cardiac output  $\dot{V}$ /min, at rest and during work

Central lines indicate regression between these parameters in control material. Inter rupted lines indicate  $\pm$  standard error of estimate. The control material has been collected by Holmgren (personal communication) from results published by Holmgren et al. (1957, 1960) and Bengtård et al. (1960).

Fig. 11 A shows the conditions for females 20-49 years old, Fig. 11 B for females 50-60 years old. Fig. 11 C shows the conditions for males 20-49 years old, Fig. 11 D for males 50-60 years old. Symbols in Fig. 7. For one symbol there was no room in Fig. 11 D. This symbol (see trace in the figure) represent cardiac output = 10.8 L/min and oxygen uptake = 1460 ml/min STPD.

with that during a resting state — by an average of 18 ml and 10 ml to 90 ml and 71 ml respectively. This increase was significant for both sexes ( )

For the men and women who performed the second work load test, i.e. 600 and 400 kpm/min respectively no noteworthy change in the stroke vol-

ume was found on comparison with the first load. For the men there was a mean increase in stroke volume of 4 ml and for the women a mean decrease of 1 ml. When however the men were classified into sickness point groups it was found that for those who had fewer than 1000 points (group A) the stroke volume during exercise at 300 kpm/min was on an average 19 ml higher than that at rest and the stroke volume during exercise at 600 kpm/min was on an average 27 ml higher than the resting value. The corresponding increases for the men who attained 2000 points or more (group C) were 12 and 15 ml respectively. The difference between the increase in stroke volume for group A and that for group C was not however significant. For the women, no noteworthy difference was found between the different sickness point groups. The relation between stroke volume/blood volume and heart rate is shown in Figure 12.

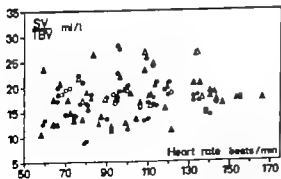


Fig 12 Stroke volume (SV) divided by total blood volume (TBV) ml/l in relation to heart rate beats/min at rest and during work. Symbols as in Fig 8.

The influence of different case history factors on the variables used for measuring hemodynamic or respiratory function

For each of the following variables: mean pressure in the pulmonary artery during exercise, mean pressure in the pulmonary artery during anoxic anoxia, arterial oxygen saturation during exercise, arterial oxygen saturation during anoxic anoxia, pulmonary vascular resistance during exercise, forced expiratory volume during one second at rest, ratio of residual volume to total lung capacity.

A selection was made from 56 patients, i.e. those men and women who during heart catheterization performed exercise at 300 and 200 kpm/min respectively. In order to obtain the best contrast between two groups of patients, a selection was made from the 56 patients of firstly the 20 who showed the greatest pathological deviation from normal values, and secondly the 20 who deviated most greatly in the opposite direction.

The aim was to study, for each of the variables, the difference between these two groups of patients with regard to each of the case-history factors given on page 41.

In neither group of patients did the observed ratio of men/women differ significantly from the predicted 50:50 ratio. It was therefore taken into account in the subsequent analyses.

The estimated degree of influence of

the different case-history factors on each of the function variables will be given below

*Mean pressure in the pulmonary artery during exercise*

The 20 patients who were recorded as having the highest pressures had a mean value of 30.1 mm Hg (range 23—63) while the mean value of those with the lowest pressures was 14.7 mm Hg (range 8—17).

The difference between the two groups was found to be significant ( ) for age, age at onset and the estimated mean annual sickness points for the period 1937—61. Those with a higher age, those with a higher age at onset and those with a higher number of sickness points during this later 5-year period showed on an average a relatively high mean pressure in the pulmonary artery during exercise. No significant differences were found for the estimated total sickness points, duration of the asthma, estimated number of attack hours or number of admissions to hospital on account of asthma.

*Mean pressure in the pulmonary artery during anoxic anoxia*

The 20 patients who were recorded as having the highest pressures had a mean value of 25.6 mm Hg (range 18—47) while the mean value of those with the lowest pressures was 11.8 mm Hg (range 9—14).

For none of the case-history factors studied here was there any significant difference between the two groups.

*Arterial oxygen saturation during exercise*

The mean value for the 20 patients

for whom the lowest oxygen saturation values in the brachial artery were obtained was 92.4 % (range 82.6—93.0). The corresponding value for the 20 patients with the highest values was 98.4 % (range 97.6—99.9).

The difference between the two groups of patients was statistically significant ( ) for age and age at onset. Those patients with a higher age and those with a higher age at onset showed on an average a relatively low arterial oxygen saturation during exercise. No significant difference was found for the other case-history factors.

*Arterial oxygen saturation during anoxic anoxia*

The 20 patients for whom the lowest oxygen saturation values in the brachial artery were obtained had a mean value of 66.7 % (range 53.4—75.3). The corresponding value for the 20 patients with the highest saturation values was 84.7 % (range 79.1—95.1).

The difference between the two groups of patients was found to be significant ( ) both for age and for the number of admissions to hospital on account of bronchial asthma. Those with a higher age and those with a higher number of hospital admissions (26) showed on an average a relatively low oxygen saturation value in the brachial artery during anoxic anoxia. No significant difference was found for the other case-history factors.

*Pulmonary vascular resistance (R) during exercise*

The 20 patients for whom the highest vascular resistance was recorded

ume was found on comparison with the first load. For the men there was a mean increase in stroke volume of 4 ml and for the women a mean decrease of 1 ml. When, however, the men were classified into sickness point groups it was found that for those who had fewer than 1000 points (group A) the stroke volume during exercise at 300 kpm/min was on an average 10 ml higher than that at rest and the stroke volume during exercise at 600 kpm/min was on an average 27 ml higher than the resting value. The corresponding increases for the men who attained 2000 points or more (group C) were 12 and 15 ml respectively. The difference between the increase in stroke volume for group A and that for group C was not however significant. For the women no noteworthy difference was found between the different sickness point groups. The relation between stroke volume/blood volume and heart rate is shown in Figure 12.

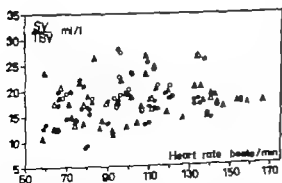


Fig. 12 Stroke volume (SV) divided by total blood volume (TBV) ml/l in relation to heart rate beats/min, at rest and during work.

Symbols as in Fig. 8.

## The influence of different case history factors on the variables used for measuring hemodynamic or respiratory function

For each of the following variables mean pressure in the pulmonary artery during exercise  
mean pressure in the pulmonary artery during anoxic anoxia  
arterial oxygen saturation during exercise,  
arterial oxygen saturation during anoxic anoxia  
pulmonary vascular resistance during exercise,  
forced expiratory volume during one second at rest  
ratio of residual volume to total lung capacity

a selection was made from 56 patients: i.e. those men and women who during heart catheterization performed exercise at 300 and 200 kpm/min respectively. In order to obtain the best contrast between two groups of patients, a selection was made from the 56 patients of firstly the 20 who showed the greatest pathological deviation from normal values, and secondly the 20 who deviated most greatly in the opposite direction.

The aim was to study for each of the variables, the difference between these two groups of patients with regard to each of the case-history factors given on page 41.

In neither group of patients did the observed ratio of men/women differ significantly from the predicted. No regard was therefore taken of sex in the subsequent analyses.

The estimated degree of influence of



had a mean value of  $R=2.42$  (range 1.67—6.28). The 20 patients with the lowest vascular resistance had a mean value of  $R=0.96$  (range 0.50—1.33).

The difference between the two groups of patients was significant (\*) both for age and for age at the onset of asthma. Those with a higher age and those with a higher age at onset showed on an average a relatively high pulmonary vascular resistance during exercise. No significant difference was found for the other case history factors.

#### *Forced expiratory volume during one second ( $FEV_{1s}$ ) at rest*

The mean value for the 20 patients for whom the lowest  $FEV_{1s}$  values, as a percentage of the predicted value were recorded (note that each patient is represented by the highest value that he or she was able to produce) was 50.7 % (range 30.8—63.3). The 20 patients with the highest values had a mean value of 90.2 % (range 77.3—110.3) of the predicted value.

The difference between the two groups of patients was significant (\*) for age, the estimated total number of attack hours and the estimated mean annual number of sickness points during the period 1957—61. Those with a higher age, those with a higher number of sickness points during 1957—61 and those with a greater number of attack hours showed on an average a relatively low forced expiratory volume during one second. No significant difference was found for the other case history factors.

#### *Ratio of residual volume to total lung capacity*

The mean value for the 20 patients with the highest  $RV/TLC$  ratio as a percentage of the predicted value, was 170.9 % (range 153.9—200.0). The patients who were recorded as having the 20 lowest values had a mean value of 114.8 % (range 88.8—132.6) of the predicted value.

For none of the case history factors studied was any significant difference observed between the two groups. There was a marked tendency however for those with a higher  $RV/TLC$  ratio to have on an average a higher total number of sickness points, but the difference between the two groups of patients did not reach the significance level.

---

To summarize the results of this study on the influence of these case history factors on variables measuring hemodynamic or respiratory function, it may be said that for several of these variables some factors were found to be of statistically significant importance. This was valid for mean pressure in the pulmonary artery during exercise, arterial oxygen saturation during exercise and during anoxic anoxia, pulmonary vascular resistance during exercise and forced expiratory volume during one second at a resting state. The variables for which no factor was found to be of significance were the mean pressure in the pulmonary artery during anoxic anoxia and the ratio of residual volume to total lung capacity. With regard to the former group of variables, it was common to all that age

had a statistically significant influence. Other case history factors with a similar degree of influence on one or more of the variables were the estimated mean annual number of sickness points during the five years almost immediately preceding the investigation, the estimated total number of attack hours, the number of admissions to hospital on account of bronchial asthma and the age at onset of the disease.

### Study of the relationship between pairs of variables measuring hemodynamic function, ventilation capacity and lung volumes

A study was made of the relationship between different pairs of each of the variables mentioned on page 84 con-

stituting a measure of hemodynamic function, ventilation capacity or lung volumes.

The material comprised 50 patients, i.e. those men and women who during the heart catheterization performed exercise at 300 and 200 kpm/min respectively. For each of the seven variables the 20 patients showing the least favourable values were selected. The number of patients among these 20 who were common to any two variables was then noted.

Assuming that no relationships existed, the predicted number of persons in each figure in Table 35 would be 7.1. As the table shows, there was a significant relationship ( ) between the mean pressure in the pulmonary artery during relatively light muscular exercise and both the corresponding pressure during asoxic anoxia and the pulmonary vascular resistance during

	$P_{am} m.w$	$\dot{V}_{O_2} m.w$	$R_{m.w}$	$P_{am} a.a.$	$\dot{V}_{O_2} a.a.$	$FEV_{1.0}$	$RV/TLC$
$P_{am} m.w$		11	13*	13*	12	8	7
$\dot{V}_{O_2} m.w$	11		12	11	12	11	9
$R_{m.w}$	13*	12		11	11	9	10
$P_{am} a.a.$	13*	11	11		15	10	8
$\dot{V}_{O_2} a.a.$	12	12	11	15		9	7
$FEV_{1.0}$	8	11	9	10	9		8
$RV/TLC$	7	9	10	8	7	8	

Tbl. 35. For pairs of variables the number of patients out of the 20 showing the least favourable values for each of the variables. The degree of significance is assessed.

$P_{am} m.w$  — mean pressure in pulmonary artery during light muscular work  
 $P_{am} a.a.$  — — — — — during asoxic anoxia  
 $\dot{V}_{O_2} m.w$  — oxygen saturation in brachial artery during light muscular work  
 $\dot{V}_{O_2} a.a.$  — — — — — during asoxic anoxia  
 $R_{m.w}$  — pulmonary vascular resistance during light muscular work  
 $FEV_{1.0}$  — forced expiratory volume in one second, 1 rest  
 $RV/TLC$  — residual volume in relation to total lung capacity, 1 rest



exercise. A similarly significant relationship (\*) was found between the arterial oxygen saturation and the mean pressure in the pulmonary artery in both cases during anoxic anoxia. With regard to the other pairs of variables the observed number of persons common to both variables in each pair exceeded the predicted number. There was an exception however as regards the relationship between the ratio  $RV/TLC$  and both the mean pressure in the pulmonary artery during muscular work and during anoxic anoxia and the arterial oxygen saturation during anoxic anoxia.

#### Patients with a chronic disease in addition to bronchial asthma

Of the 68 patients below the age of 60 years, who underwent heart catheterization six (three men and three women) had another chronic disease in addition to bronchial asthma, which it was considered might have had a decisive influence on the cardio-respiratory functions. These patients who were not included in the previous analyses of the results obtained during catheterization are reported in Table 36.

For these six patients the experimental conditions were the same as for other

	No	Heart rate beats/min	O <sub>2</sub> saturation per cent		AVD ml/l	O uptake ml/min STPD	Cardiac output l/min	Stroke volume ml	PA M	PCV M	Br A M	R
			Br A	PA								
Rest	6											
mean		94	93	87	47	230	5.5	59	15	7	105	1.6
highest		102	95	73	66	281	6.9	74	20	10	125	3.2
lowest		85	91	56	38	206	3.7	36	9	4	90	0.6
Anoxic anoxia	6											
mean		102	68	49	41	191	5.5	54	22		107	
highest		111	74	56	50	257	6.4	66	26		135	
lowest		94	60	36	30	173	4.8	43	13		62	
Work I	6											
mean		126	91	48	78	740	12.5	96	30	12	132	1.8
highest		143	94	76	96	1007	29.8	210	35	15	163	3.6
lowest		112	87	22	29	685	7.7	56	24	6	90	0.7
Work II	2											
mean		164	91	34	103	1342	13.1	84	32	13	138	1.4
highest		186	92	38	114	1837	16.3	98	33	14	163	1.4
lowest		143	91	39	96	1527	9.9	70	28	12	150	1.4

Table 36. Observations during heart catheterization in 6 patients (3 men and 3 women) who suffered from a chronic disease in addition to bronchial asthma.

Work I = 300 kpm/min for males, 200 kpm/min for females. Work II = 600 kpm/min for males, 400 kpm/min for females. Abbreviations as in Table 37.

patients, with the exception of the fact that one of the men performed 180 kpm/min at the first work load, compared with 300 kpm/min performed by the other men. Since these patients were so small in number the results were analysed regardless of sex and age.

On comparison of the mean values for the different function variables (Table 36) with the corresponding figures for the other patients (Tables 37

—4) considerable differences were found for some of the variables. These differences were not, however statistically significant. It should be mentioned that none of the patients showed signs of pulmonary hypertension. This group of patients was so small, however and the supervening diseases so varied that no conclusions were drawn.

Groups acc. to sickness points and age (yrs.)	Charac- teristic	No	Heart rate/ min	Oxygen saturation per cent		A/D ml/l	Oxygen uptake ml/min STPD
				Br A	PA		
A	30-49	3	61	97.4	74.7	46.4	233
	50-59	6	72	94.6	67.8	48.7	226
	Total A	9	66	95.5	70.1	47.9	235
B	30-49	5	73	93.5	72.8	42.7	216
	50-59	2	75	96.4	69.6	48.9	204
	Total B	7	74	93.8	71.9	44.5	224
C	30-49	5	72	96.2	76.6	43.4	226
	50-59	4	87	95.0	68.3	50.1	236
	Total C	9	79	96.8	77.9	45.8	239
Total							
A+B+C	30-49	13	71	97.0	74.7	43.4	210
"	50-59	12	78	95.0	68.3	49.2	232
Total							
A+B+C	30-59	25	74	96.0	71.6	46.2	236
" "	mean		92	99.7	78.6	59.7	263
	highest		56	87.0	60.6	37.0	178
	lowest		11	31.2	5.1	5.9	33
	SE		2.3	0.6	1.1	1.2	6.5

Table 3. Observations during heart catheterization at rest in male patients  
No. = 25 except for PCV and R No. = 23

Group A	<1000 sickness points	PCV	= pulmonary capillary venous pressure
B	1000-1999	RA	= right atrium
C	≥2000	S	= systolic pressure
Br A	= brachial artery	D	= diastolic pressure
PA	= pulmonary artery		

Groups acc. to sickness points and age (yrs.)	Charac- teristic	No	Heart rate, beats/ min	Oxygen saturation per cent		A/D ml/l	Oxygen uptake ml/min STPD
				Br A	PA		
A	30-49	7	78	96.5	72.2	42.9	203
	50-59	4	72	97.5	70.6	43.0	186
	Total A	11	6	96.0	71.6	42.7	197
B	30-49	9	80	96.2	72.8	42.3	219
	50-59	7	79	98.0	68.3	51.4	213
	Total B	16	80	97.0	70.6	46.8	211
C	30-49	6	79	96.7	72.1	42.2	215
	50-59	5	85	93.4	69.5	43.3	181
	Total C	11	82	96.1	71.1	42.7	200
Total							
A+B+C	30-49	22	79	96.4	71.4	42.3	209
"	50-59	16	79	97.1	69.3	47.3	196
Total							
A+B+C	30-59	38	79	96.7	71.1	43.5	204
" "	mean		100	100.0	81.4	57.9	267
	highest		59	91.7	63.2	29.7	122
	lowest		12	2.3	4.5	6.8	29
	SE		1.9	0.4	0.7	1.1	4.7

Table 3A. Observations during heart catheterization at rest in female patients.  
No. = 38 except for PCV and R No. = 37 Abbreviations as in Table 3

Cardiac output l/min	Stroke volume ml	Pressure, mm Hg									Pulm. vase- resist. (R)	Pulm. asc- resist. index (R <sub>i</sub> )
		RA	PA			PCV	Br A					
			M	S	D		M	S	D	M		
5.8	89	1.0	17	7	11	5	130	78	98	1.32	2.41	
4.8	87	-0.5	22	8	13	6	151	83	113	1.81	3.13	
8.1	74	0	20	8	12	6	133	81	106	1.66	3.06	
5.8	81	1.1	17	8	11	5	140	80	104	1.21	2.36	
4.2	87	2.5	19	9	12	4	178	90	120	1.94	3.37	
5.3	74	1.7	18	8	11	5	145	82	104	1.43	2.56	
5.4	75	-1.3	16	8	10	3	132	71	91	1.39	2.41	
5.2	86	-0.5	23	7	14	4	145	81	109	1.83	3.40	
5.3	86	-0.9	19	6	12	3	136	75	101	1.37	2.85	
5.8	81	0.3	17	6	11	4	135	76	100	1.37	2.35	
4.8	83	0	23	8	13	5	161	83	112	1.68	3.13	
5.2	72	0.3	19	7	13	5	142	79	106	1.49	2.81	
7.6	124	5	31	18	30	8	170	110	140	2.77	5.14	
5.1	45	-3	13	3	7	1	190	60	80	0.60	0.81	
1.1	17	1.9	8	2.9	3	2.4	17.3	13.1	17	0.72	1.38	
0.2	3.4	0.4	0.9	0.6	0.7	0.6	3.6	2.7	3.6	0.15	0.37	

M = mean pressure  
 AVD = aortic-ventricular  
 oxygen difference  
 R =  $\frac{P_{PA} - P_{PCV}}{Q}$

$$R_i = \frac{P_{PA} - P_{PCV}}{Q} \text{ BSA}$$

Cardiac output l/min	Stroke volume ml	Pressure, mm Hg									Pulm. vase. resist. (R)	Pulm. vase. resist. index (R <sub>i</sub> )
		RA	PA			PCV	Br A					
			M	S	D		M	S	D	M		
4.8	63	0.7	17	7	11	7	137	79	98	0.85	1.60	
4.3	68	0.8	21	7	11	4	146	81	106	1.73	2.78	
4.6	62	0.6	18	7	11	6	150	80	100	1.33	2.60	
4.1	61	0.6	19	8	12	6	144	80	106	1.35	2.36	
4.2	51	0.8	20	9	12	7	171	87	121	1.24	2.10	
4.7	60	0.9	19	8	12	6	156	83	111	1.31	2.38	
5.3	67	0.3	20	8	13	4	132	73	97	1.19	2.30	
4.2	51	0.2	30	8	12	4	149	83	112	1.56	2.30	
4.8	60	0.3	30	8	12	6	140	78	101	1.73	2.82	
5.1	63	0.7	18	8	12	6	139	78	101	1.27	2.13	
4.3	55	0.5	20	8	12	5	156	80	111	1.62	2.61	
4.7	61	0.6	19	8	13	6	157	80	107	1.41	2.32	
8.0	100	1	29	14	30	11	203	100	145	2.82	4.51	
2.9	30	-1	11	2	5	1	110	53	63	0.28	0.23	
18.8	11	1.3	3	2.7	4	3.6	37.3	12.8	18	0.74	1.18	
1.8	2.3	0.2	0.7	0.4	0.6	0.4	4.1	2.1	3.0	0.12	0.19	

Groups acc. to sickness points and age (yrs.)	Charac- teristic	No	Heart rate beats/ min	Oxygen saturation per cent		AVD ml/l	Oxygen uptake ml/min STPD	
				Br A	PA			
A	30-49	mean	3	61	97.4	74.7	46.4	233
	50-59	"	6	72	91.6	67.8	48.7	226
	Total A	"	9	69	93.5	70.1	47.9	225
B	30-49	"	5	73	93.5	72.8	42.7	216
	50-59	"	2	75	96.4	69.6	48.9	204
	Total B	"	7	74	93.8	71.0	44.5	224
C	30-49	"	5	72	98.2	76.6	42.4	226
	50-59	"	4	87	93.0	68.3	50.1	236
	Total C	"	9	79	96.8	72.9	45.8	239
Total								
A+B+C	30-49		13	71	97.0	74.7	43.4	240
	50-59		12	78	93.0	68.3	49.2	232
Total								
A+B+C	30-59	mean	25	4	96.0	71.6	46.2	226
		highest		92	99.7	78.6	50.7	263
		1 west		56	87.0	60.6	37.0	178
		SD		11	31.2	5.1	3.9	33
		SE		2.3	0.6	1.1	1.3	6.6

Table 3 Observations during heart catheterization 1 test in male patients  
No = 25 except for PCV and R No = 23

Group A	<1000 sickness points	PCV	= pulmonary capillary venous pressure
B	1000-1999 "	RA	= right atrium
C	≥2000 "	S	= systolic pressure
Br A	= brachial artery	D	= diastolic pressure
PA	= pulmonary artery		

Groups acc. to sickness points and age (yrs.)	Charac- teristic	No	Heart rate, beats/ min	Oxygen saturation per cent		AVD ml/l	Oxygen uptake ml/min STPD	
				Br A	PA			
A	30-49	mean	7	78	96.5	72.2	42.9	203
	50-59		4	72	97.5	70.6	43.6	186
	Total A		11	76	96.9	71.6	43.7	197
B	30-49		9	80	96.2	72.8	42.3	210
	50-59		7	79	98.0	68.3	51.4	213
	Total B		16	80	97.0	70.8	46.3	211
C	30-49		6	79	96.7	72.1	42.2	216
	50-59		5	83	95.4	69.6	43.3	181
	Total C		11	82	96.1	71.1	42.7	200
Total								209
A+B+C	30-49		22	79	96.4	72.4	42.5	196
	50-59		16	79	97.1	69.3	47.3	
Total								204
A+B+C	30-59	mean	33	79	96.7	71.1	44.5	207
		highest		100	100.0	81.4	37.9	122
		1 west		59	91.7	63.2	29.7	29
		SD		12	2.3	4.5	3.1	4.7
		SE		1.9	0.4	0.7	1.1	

Table 38 Observations during heart catheterization 1 test in female patients  
No = 38, except for PCV and R No = 37 Abbreviation as in Table 37

Oxygen uptake ml/min STPD	Cardiac output l/min	Stroke volume ml	Pressures, mm Hg					
			PA			Br A		
			S	D	M	S	D	M
229	8.4	76	21	9	18	120	63	87
218	8.5	68	23	11	20	146	78	101
222	8.5	71	20	12	18	133	74	98
228	8.4	73	28	12	18	120	63	81
186	4.6	53	27	13	18	181	82	106
216	8.9	66	28	12	18	129	66	90
218	6.3	66	24	9	13	119	65	82
229	4.7	49	40	19	30	147	75	101
226	6.1	60	35	13	22	121	68	92
221	8.6	73	26	10	16	120	63	81
216	8.1	62	37	16	22	148	78	104
228	8.8	66	21	12	16	123	70	94
221	8.8	100	72	32	47	179	90	120
163	3.6	41	12	6	9	82	32	50
22	1.3	16	13	7.9	10	27.2	15.6	19
6.1	0.2	3.2	2.1	1.6	2.0	5.8	2.3	2.9

Oxygen uptake ml/min STPD	Cardiac output l/min	Stroke volume ml	Pressures, mm Hg					
			PA			Br A		
			S	D	M	S	D	M
180	4.3	61	25	10	16	117	65	88
167	4.3	60	21	11	16	140	81	104
17	4.3	61	27	10	17	125	71	94
191	4.7	66	26	11	17	121	71	96
186	4.1	49	26	12	16	166	80	106
180	4.6	63	26	11	17	142	75	101
162	4.6	62	26	11	18	126	69	92
163	3.9	44	21	12	16	123	69	97
173	4.2	48	20	11	17	120	69	94
18	4.5	62	26	11	17	125	68	92
174	4.1	47	29	18	17	143	77	104
180	4.2	31	27	11	17	122	72	97
267	7.1	77	43	28	32	190	100	145
132	2.7	21	16	6	8	82	50	58
20	10.3	11	7	4.6	5	21.9	12.2	17
4.9	1.7	1.6	1.1	0.7	0.8	2.8	2.8	2.8

Groups acc. to sickness points and age (yrs.)		Charac- teristic	No	Heart rate beats/ min	Oxygen saturation per cent		A/D ml/l
					Br A	PA	
A	30-49	mean	3	72	78.0	56.5	42.8
	50-59		6	82	76.0	50.7	40.7
	Total A		9	79	76.0	52.8	41.3
B	30-49	"	5	86	71.4	51.5	31.3
	50-59		2	89	75.0	52.3	41.3
	Total B	"	7	87	4.8	53.9	38.2
C	30-49		5	81	62.7	61.4	40.9
	50-59	"	4	100	70.3	43.8	49.3
	Total C		9	89	77.2	53.6	41
Total							
A+B+C	30-49	"	13	81	78.4	57.6	38.7
	50-59	"	12	89	73.4	48.7	43.7
Total							
A+B+C 30-59		mean	25	85	6.0	53.3	41.1
		highest		119	85.1	71.8	58.0
		lowest		60	34.8	33.9	30.8
		SD		14	6.8	8.9	6.7
		SE		2.8	1.8	1.8	1.2

Table 39 Observations during anoxic anoxia on heart catheterisation in male patients  
Abbreviations as in Table 37

Groups acc. to sickness points and age (yrs.)		Charac- teristic	No.	Heart rate beats/ min	Oxygen saturation per cent		A/D ml/l
					Br A	PA	
A	30-49	mean	7	83	74.9	51.5	42.0
	50-59		4	86	73.1	48.7	40.1
	Total A		11	83	74.9	50.6	41.3
B	30-49		9	84	60.7	56.1	42.9
	50-59		7	88	73.8	50.0	45.0
	Total B		16	86	72.7	52.4	43.7
C	30-49		6	88	78.2	53.2	41.8
	50-59		5	90	71.3	48.3	41.4
	Total C		11	89	75.1	50.1	41.6
Total							
A+B+C	30-49		22	83	78.6	53.8	42.3
	50-59		16	88	73.6	48.6	42.5
Total							
A+B+C 30-59		mean	38	86	76.5	51.8	42.4
		highest		153	91.5	61.3	61.2
		lowest		55	53.1	28.6	27.7
		SD		18	8.7	7.8	7.6
		SE		2.1	1.3	1.2	1.2

Table 40 Observation during anoxic anoxia on heart catheterisation in female patients  
Abbreviations as in Table 37

Stroke volume ml	Pressure, mm Hg									Pulse wave cath. (R)	Pulse wave index (R)	
	PA			PCV		Br A						
	S	D	M	No.	M	No.	S	D	M			
107	28	11	16	3	4	3	172	83	113	2	1.30	2.34
91	45	28	28	4	10	4	184	87	124	4	1.75	2.38
87	29	17	24	6	8	7	185	91	126	6	1.60	2.03
85	33	18	22	5	7	5	158	79	114	5	1.48	2.06
84	41	30	27	1	12	2	178	93	131	1	1.30	2.24
98	36	10	23	6	8	7	164	83	119	8	1.43	2.61
85	25	8	18	5	6	4	149	76	100	4	0.92	1.79
86	33	23	33	4	7	4	201	96	128	4	2.00	5.37
82	30	18	24	9	6	8	175	87	122	8	2.01	2.55
97	29	12	18	12	8	12	159	79	110	11	1.23	2.28
81	48	21	30	9	9	10	184	87	124	9	2.30	4.15
90	38	16	24	21	7	22	173	87	122	20	1.71	2.13
130	97	32	63		18		230	120	163		8.38	16.88
87	12	3	8		3		130	70	100		0.50	0.78
90	17	9.0	12		3.3		28.1	14.4	21		1.23	2.21
4.0	3.8	1.9	2.4		0.7		6.3	3.1	4.3		0.30	0.50

Stroke volume ml	Pressure, mm Hg										Pulse wave cath. (R)	Pulse wave index (R)
	PA			PCV		Br A						
	S	D	M	No.	M	No.	S	D	M			
102	28	11	17	2	4	2	183	92	128	2	1.83	1.84
107	47	21	33	4	10	4	218	103	151	2	1.57	2.90
183	40	17	27	6	8	7	208	98	141	8	1.25	2.54
91	39	13	23	4	8	3	181	86	118	4	1.20	1.66
72	43	23	33	0		1	223	105	150	0		
99	39	17	25	4	8	6	168	89	123	4	1.30	1.56
83	30	10	17	4	6	4	184	86	113	3	0.84	1.70
86	43	11	23	9		1	180	75	110	0		
88	32	18	18	4	6	3	167	83	112	3	0.94	1.79
95	25	12	18	10	6	12	179	87	119	9	1.06	1.1
96	46	30	33	4	10	8	215	98	144	3	1.57	2.09
90	38	15	23	14	7	18	100	81	127	12	1.20	2.03
147	74	34	82		13		236	115	170		2.14	4.56
81	17	5	6		3		136	70	100		0.16	0.30
27	14	7.8	11		3.6		28.6	13.8	21		0.60	1.12
8.2	3.1	1.7	2.8		1.0		7.9	3.7	6.7		0.17	0.32



Groups acc. to sickness points and age (yrs)		Charac- t ristic	No.	Heart rate, beats/ min	Oxygen saturation per cent		AVD ml/l	Oxygen uptake ml/min STPD	Cardiac output l/min
					Br A	PA			
A	30-49	mean	3	93	96.4	51.0	92.7	830	9.7
	50-59	"	5	100	93.2	41.9	96.5	867	9.1
	Total A	"	8	107	94.4	45.8	95.1	872	9.3
B	30-49		5	108	94.9	47.4	88.7	883	10.9
	50-59		2	91	91.8	41.7	94.8	869	9.2
	Total B		7	103	91.0	46.6	90.4	880	9.8
C	30-49		5	98	96.7	56.1	82.6	710	9.0
	50-59	"	4	124	91.7	37.3	104.4	854	8.1
	Total C		9	110	93.6	47.7	92.3	791	8.6
Total									
A+B+C	30-49	"	13	101	96.7	51.0	87.3	826	9.3
"	50-59	"	11	107	93.0	40.7	99.1	863	8.8
Total									
A+B+C	30-50	mean	24	104	95.0	46.6	92.7	844	9.2
"	"	highest		140	90.9	60.7	111.2	1016	12.1
"	"	lowest		78	82.6	27.8	71.0	510	6.6
"	"	SD		14	4.3	8.6	10.6	108	1.4
"	"	SE		2.8	0.9	1.8	2.3	22.1	0.3

Table 41 Observations on heart catheterization during work 300 kpm/min in male patients.  
Abbreviations as in Table 37

Groups acc. to sickness points and age (yrs.)	Charac- teristic	No	Heart rate/ min	Oxygen saturation per cent		AVD ml/l	O ygen uptake ml/min STPD	Cardiac output l/min	
				Br A	PA				
A	30-49	mean	3	129	96.2	42.0	113.6	1314	12.3
	50-59		5	120	90.3	33.9	101.8	1393	12.7
	Total A		8	120	92.5	36.9	108.1	1375	13.1
B	30-49		5	136	92.9	36.6	107.3	1368	12.8
	50-59		1	158	90.7	27.5	132.9	1496	11.3
	Total B		6	140	93.5	35.1	111.6	1380	13.6
C	30-49		5	130	96.8	44.1	110.0	1383	12.9
	50-59		1	183	97.5	32.3	138.1	1297	10.1
	Total C		6	134	98.6	42.1	113.0	1366	12.3
Total									
A+B+C	30-49		13	132	95.9	40.7	109.8	1367	12.7
	50-59		7	137	92.4	32.8	112.1	1394	12.8
Total									
A+B+C	30-50	mean	20	134	94.8	37.9	110.0	1377	12.7
		highest		166	100.0	40.7	133.4	1505	19.5
		lowest		100	74.3	24.0	74.4	1069	8.9
		SD		17	6.3	7.2	20.0	114	2.4
		SE		3.7	1.4	1.6	4.4	4.1	0.5

Table 42 Observations on heart catheterization during work 600 kpm/min, in male patients.  
Abbreviations as in Table 37

Stroke volume ml	Pressures, mm Hg									Pulm. vasc. resist. (R)	Pulm. vasc. resist. index (R)	
	PA			PCV		B A			No.			
	S	D	M	No.	M	No.	S	D				M
73	26	11	18	5	6	6	163	85	118	5	1.54	2.51
61	43	17	26	4	8	3	187	89	135	4	2.24	3.63
68	24	12	21	9	7	9	171	86	121	9	1.65	3.01
73	36	14	20	5	9	6	165	88	126	5	1.31	2.14
70	32	13	20	4	9	5	207	90	143	4	1.29	2.26
72	31	14	20	8	9	11	189	93	134	9	1.30	2.19
78	25	15	21	5	9	5	167	83	115	4	1.80	2.54
61	27	14	22	2	9	4	200	93	139	2	1.87	2.77
72	26	15	22	7	9	9	182	87	126	6	1.62	2.82
73	31	13	20	13	8	17	166	86	120	14	1.53	2.47
60	36	15	23	10	9	12	200	94	140	10	1.70	2.91
71	33	14	21	23	8	29	168	89	128	24	1.64	2.65
100	48	27	33		15		245	119	170		3.29	4.94
83	20	8	10		4		120	65	93		0.80	0.88
13	8	4.5	6		2.8		35.0	12.8	22		0.75	1.18
2.3	1.4	0.9	1.1		0.5		6.8	2.4	4.1		0.15	0.23

Stroke volume ml	Pressures, mm Hg									Pulm. vasc. resist. (R)	Pulm. vasc. resist. index (R)	
	PA			PCV		Br A			No.			
	S	D	M	No.	M	No.	S	D				M
77	36	13	21	8	9	7	178	91	122	6	1.26	2.07
70	45	15	28	2	13	3	203	94	128	2	1.78	2.89
73	39	14	23	8	10	10	186	92	137	8	1.30	2.29
77	35	13	22	7	9	7	195	94	129	7	1.17	1.94
70	34	11	19	4	9	4	208	96	140	3	1.33	2.13
7	33	14	21	11	9	11	200	93	134	10	1.19	2.00
74	24	16	21	4	9	3	179	85	120	4	0.83	1.31
65	31	15	22	1	10	2	185	95	125	1	1.87	2.79
71	35	15	22	5	9	7	181	88	124	5	1.43	1.81
8	25	14	21	17	9	19	185	91	124	17	1.12	1.84
80	28	13	22	7	18	9	202	96	135	6	1.33	2.51
74	36	14	21	24	9	35	198	93	129	23	1.32	2.82
100	53	23	31		18		270	120	180		2.13	3.08
62	30	7	11		1		132	70	92		0.50	0.72
13	8	4.5	6		2.8		32.3	12.7	21		0.50	0.83
2.3	1.4	0.9	1.1		0.6		8.1	2.4	3.6		1.05	0.17

Groups acc. to sickness points and age (yrs.)	Charac- teristic	No	Heart rate beats/ min	Oxygen saturation per cent		AVD ml/l	Oxygen uptake ml/min STPD	Cardiac output l/min
				Br A	PA			
A 30-49	mean	6	108	96.3	48.6	85.5	686	8.0
50-59	"	4	114	96.0	39.5	97.4	670	8.8
Total A	"	10	110	96.7	45.0	90.9	679	7.8
B 30-49	"	6	107	96.0	47.0	88.1	680	7.6
50-59	"	6	115	94.4	42.9	90.3	740	8.0
Total B	"	12	110	95.2	45.1	89.3	705	7.8
C 30-49	"	6	115	97.0	50.1	82.0	721	8.8
50-59	"	4	123	94.8	42.7	90.4	712	7.9
Total C	"	10	118	96.7	47.1	85.4	717	8.5
Total A+B+C 30-49	"	18	110	96.9	48.6	85.6	697	8.2
50-59	"	14	117	95.1	41.8	92.5	712	7.6
Total A+B+C 30-59	mean	32	113	96.1	45.7	88.5	701	8.0
"	highest		141	98.3	55.6	104.2	964	11.9
"	lowest		81	91.2	31.6	69.6	522	6.2
"	SD		14	5.0	5.7	8.9	101	12.6
"	SE		2.6	0.9	1.0	1.6	17.8	2.3

Table 43. Observations on heart catheterization during work, 100 kpm/min, in female patients. Abbreviations as in Table 37

Groups acc. to sickness points and age (yrs.)	Charac- teristic	No	Heart rate beats/ min	Oxygen saturation per cent		AVD ml/l	Oxygen uptake ml/min STPD	Cardiac output l/min
				Br A	PA			
A 30-49	mean	7	122	96.4	41.9	93.3	1071	10.8
50-59	"	3	142	97.3	35.1	108.4	929	8.5
Total A	"	10	136	96.7	39.0	102.0	1028	10.1
B 30-49	"	7	135	97.1	41.1	101.7	1016	10.0
50-59	"	5	141	96.9	34.7	102.9	1058	9.6
Total B	"	12	138	97.0	38.0	102.1	1030	9.9
C 30-49	"	4	140	96.9	40.0	93.8	1050	10.9
50-59	"	2	159	93.2	33.4	109.1	1111	10.2
Total C	"	6	152	95.7	37.8	102.2	1076	10.7
Total A+B+C 30-49	"	18	141	96.6	41.2	100.2	1045	10.6
50-59	"	10	139	96.3	34.6	106.1	1027	9.3
Total A+B+C 30-59	mean	28	140	96.6	38.9	102.1	1030	10.1
"	highest		171	98.7	47.1	116.9	1416	13.1
"	lowest		100	92.4	23.8	79.6	752	6.1
"	SD		16	1.4	5.0	10.6	121	12.6
"	SE		3.0	0.3	1.1	2.0	23.3	2.4

Table 44. Observations on heart catheterization during work, 300 kpm/min, in female patients. Abbreviations as in Table 37

the impression of the author that the patients in the asthma series adopted a particularly positive attitude to the investigations and attempted to show that it was in their own interest to produce the maximum achievement possible.

For this series of patients regarded as a whole the lung volumes vital capacity and total lung capacity were on an average significantly smaller than predicted. In this respect, there was no sex difference of any notable order of size. The relatively low total lung capacity may have some connection with the reduction in vital capacity which was an average finding. The reduction in vital capacity may be explained as partly due to hyperinflation, as suggested by the relatively large residual volume this may be connected with the fact that these patients — regarded as a group — seemed to be less physically active than is usual.

Several authors have emphasized the poor correlation between vital capacity and pulmonary disease. It is not uncommon, especially in patients with chronic obstructive pulmonary diseases, to find a relatively high value for the vital capacity while at the same time the ventilation capacity is found to be markedly reduced (Baldwin, Courmand and Dickinson, 1949). Different authors have obtained different ideas of the order of size of the vital capacity in patients with chronic obstructive pulmonary disease. Hurtado, Kalreider Frye Brooks and McCann (1934) Broke Fowler and Comroe (1951) and Park and Lee (1961) generally found reduced values in these

patients, while Herschfus, Bresnick and Segal (1953) found a mean value somewhat exceeding the predicted value. A significantly higher value for both the ratios  $FRC/TLC$  and  $RV/TLC$  was found on an average in both sexes in the present asthma series on comparison with the control material. On classification of the asthma series into groups according to the over all degree of severity of the disease it was found that on an average the female groups with a high total number of sickness points attained significantly higher values than the group with a low number. This applied both to  $FRC/TLC$  and  $RV/TLC$ . Some difference was also found for the men, but this was not significant. On assessing these ratios consideration should be taken of the fact that the total lung capacity value for the material as a whole — and also when classified into groups according to sickness points — was lower than the predicted value. The results obtained in these tests thus indicate that there was a certain degree of relationship, as expected, between the over-all degree of severity of the disease and the size of the ratio of both the residual volume and the functional residual capacity to the total lung capacity.

The relationship between asthma and pulmonary emphysema has long been discussed. According to certain clinical observations asthma often leads to the development of pulmonary emphysema (Abbott, Hopkins, van Flait and Robinson, 1953 *inter alia*). Some histological investigations of patients with asthma (Gough, 1952; Williams and Leopold, 1959 among

The degree of severity of the bronchial asthma in this series of patients varied greatly and it was expected that any secondary effects on the cardiac and pulmonary functions might also vary. The patients were therefore classified into groups according to several different principles meant to represent the degree of severity of the disease as found in the case histories. The investigation findings were then compared between these groups. A further aim was to compare the asthma series — or groups of the series — with suitable control material. In the comparisons concerning ventilation capacity and lung volumes the control material used was that reported by Berglund, Birath, Bjure, Grimby, Kjellmer, Sandqvist and Söderholm (1963), Birath, Kjellmer and Sandqvist (1963) and Grimby and Söderholm (1963). The comparisons with regard to physical work capacity were made with a control material comprising individuals from the City of Uppsala Health Survey of 1961 (Irnell and Linder to be published). The hemodynamic investigation findings from the asthma material were compared in several cases with the results of a study by Holmgren, Jonsson and Sjöstrand (1960).

For comparisons with regard to respiratory function the choice of the control material lay primarily between that mentioned above and the series studied by Kory, Callahan, Borén and Syner (1961). The deciding factors for the choice of the former were as follows:

- 1 The investigations were based on Swedish persons
- 2 In principle the same apparatus and same mode of procedure were used as in the present study
- 3 The study treated both ventilation capacity and lung volumes
- 4 There was a wide age range among the group of persons studied
- 5 The material appeared to be relatively representative of the general population.

Of especial importance was the fact that the apparatus and mode of procedure were in principle the same in the present study of the asthma series and the study of the control subjects.

Apart from these factors, however, the degree of motivation in both the subject and the investigator appears to be of importance. In assessing the extent to which normal subjects have co-operated, a comparison between two independent function studies may be of value. In this control the high degree of correlation between the two tests  $FEV_{1.0}$  and  $MVV_1$  may be utilized. This relationship should however depend on the condition that the respiration is not impaired by variable air way obstruction. The strain on the subject, especially in the test of the maximum ventilation capacity leads in several patients with bronchial asthma to the provocation of some degree of respiratory tract obstruction, even when the test is performed at an optimal period with respect to the asthma. No definite objective measure of the degree of motivation could be obtained, but it is

according to the estimated degree of severity of the disease. These results thus support the view that there was some degree of relationship, as expected, between the estimated degree of severity and the ventilation capacity value found.

The degree of deviation between the observed and predicted values should be regarded with some caution, however, since there is no available control material that simultaneously fulfils all the criteria of "normal" material in the true sense of this word, which include the demand for a completely random selection of subjects from the total population.

It is probable that the differences found between the asthma series and the control material can be explained to a certain extent by the fact that the methods were not identical in practical use although identical in principle. The differences between the mean values for the two series were, however, of a considerable order of size, and it was also found that with regard to the variables for which the difference was greatest there was also a considerable difference between group A and group C, i.e. between the group that was estimated by the number of sickness points to have the greatest degree of severity since the onset of the asthma, and the group with the lowest degree.

On finding the mean deviation from the predicted value for each of the tests  $FEV_{10}$ ,  $FEV_{25}$ ,  $MVV_{40}$  and  $MVV_{15}$  for the whole main asthma series, it was found that the deviation for the latter two was the greatest, i.e. about 40 percent units. The cor-

responding deviations for  $FEV_{10}$  and  $FEV_{25}$  were about 30 and 20 percent units respectively. This supports the view that a test of maximum ventilation capacity is to be preferred for an assessment of the reduction in ventilatory function in a material such as this. The possible advantage to the subject in being able to choose his own respiratory frequency did not appear to be greater for the patients in the asthma series than for the control persons. Regardless of whether a fixed frequency of 40 breaths per minute was used in the maximum ventilation capacity test or whether the patient himself chose the frequency the asthma patients on an average attained practically the same percentage of the predicted value. Thus for the asthma patients one of these two tests was probably adequate for assessing the influence of the disease on the ventilatory function. This assumption is supported by the fact that when a selection was made from the 110 asthma patients of firstly the 20 patients with the least favourable values at the  $MVV_{40}$  test and secondly the 20 patients with the least favourable values at the  $MVV_{15}$  test, no fewer than 1 were common to each group. This indicated a highly significant relationship between these two tests of ventilation capacity.

The somewhat smaller mean difference from the control material shown by the  $FEV_{10}$  test, compared with the  $MVV$  tests, is probably connected, *inter alia* with the smaller degree of physical exertion associated with the performance of this test. The even smaller difference with regard to  $FEV_{25}$  is due

others) however have contradicted the view that the incidence of emphysema is higher in patients with asthma. Gloor (1954) arrived at the opposite conclusion. This contradiction may be explained in the words of Kemp (1961): "Wir glauben, dass die Diskrepanz zwischen klinischen und histopathologischen Ergebnissen zum Teil auf einer unterschiedlichen Auslegung des Emphysembegriffes beruht. Viele Kliniker bezeichnen jede Lungenüberblähung zumal wenn sie längere Zeit besteht als Emphysem während von pathologisch anatomischer Seite das Vorhandensein bestimmter morphologischer Merkmale für die Diagnose eines Emphysems im engeren Sinne gefordert wird."

An increased ratio of FRC/TLC and RV/TLC will be subsequently referred to in this discussion as "hyperinflation" and will be regarded as being consistent with but not specifically evidential of emphysema.

In comparison with the control material the asthma series as a whole showed on an average a significantly reduced ventilation capacity. Large variations were found in this series for each of the different tests. These variations however were observed not only for the material as a whole but also within the groups formed according to factors representing the estimated degree of severity of the disease, such as the number of sickness points, the duration etc. One possible reason is that the patients — in spite of the fact that in reality their asthma was of similar degrees of severity — exhibited considerable differences in ventilation capacity. This could be due to individ-

ual dissimilarities, for example the difference in the capacity of the lung tissue to resist strain of the type caused by bronchial constriction. The different errors of the method also play a role. The error in estimating the degree of severity of the disease, for example, is an important factor in the analysis of the results. The fact that an error of method exists is due not only to lack of memory and expressive ability on the part of some patients, but also to the fact that different patients do not measure the degree of severity in the same way and experience their symptoms differently even when these are in principle of the same type and grade. Another factor of considerable importance was the difficulty in ascertaining that the subjective condition of a patient at the time of an investigation was optimal with respect to the asthma. These factors, together with others such as motivation, muscular strength, degree of training etc., contribute to the relatively wide ranges in the ventilation capacity and lung volumes even in groups of patients in whom the asthma was assessed as having been of a relatively similar degree of severity. This line of reasoning obviously holds in principle for the physical work capacity and hemodynamic functions also.

When the mean observed values for  $FEV_{1.0}$ ,  $FEV_x$ ,  $MVV_{25}$  and  $MVV_{50}$  were calculated separately for the asthma material as a whole, a highly significant difference was found from the mean predicted values. For each of these tests significant differences were also shown between the different groups on classification of the material.

according to the estimated degree of severity of the disease. These results thus support the view that there was some degree of relationship as expected, between the estimated degree of severity and the ventilation capacity value found.

The degree of deviation between the observed and predicted values should be regarded with some caution, however, since there is no available control material that simultaneously fulfils all the criteria of "normal" material in the true sense of this word, which include the demand for a completely random selection of subjects from the total population.

It is probable that the differences found between the asthma series and the control material can be explained to a certain extent by the fact that the methods were not identical in practical use although identical in principle. The differences between the mean values for the two series were however of a considerable order of size, and it was also found that with regard to the variables for which the difference was greatest there was also a considerable difference between group A and group C, i.e. between the group that was estimated by the number of sickness points to have the greatest degree of severity since the onset of the asthma, and the group with the lowest degree.

On studying the mean deviation from the predicted value for each of the test  $FEV_{1.0}$ ,  $FEV_{1.5}$ ,  $MVV_{50}$  and  $MVV$  for the whole main asthma series, it was found that the deviation for the latter two was the greatest, i.e. about 40 percent units. The cor-

responding deviations for  $FEV_{1.0}$  and  $FEV_{1.5}$  were about 30 and 20 percent units respectively. This supports the view that a test of maximum ventilation capacity is to be preferred for an assessment of the reduction in ventilatory function in a material such as this. The possible advantage to the subject in being able to choose his own respiratory frequency did not appear to be greater for the patients in the asthma series than for the control persons. Regardless of whether a fixed frequency of 40 breaths per minute was used in the maximum ventilation capacity test or whether the patient himself chose the frequency the asthma patients on an average attained practically the same percentage of the predicted value. Thus for the asthma patients one of these two tests was probably adequate for assessing the influence of the disease on the ventilatory function. This assumption is supported by the fact that when a selection was made from the 116 asthma patients of firstly the 20 patients with the least favourable values at the  $MVV_{50}$  test, and secondly the 20 patients with the least favourable values at the  $MVV$  test, no fewer than 17 were common to each group. This indicated a highly significant relationship between these two tests of ventilation capacity.

The somewhat smaller mean difference from the control material shown by the  $FEV_{1.0}$  test, compared with the  $MVV$  tests, is probably connected, *inter alia* with the smaller degree of physical exertion associated with the performance of this test. The even smaller difference with regard to  $FEV_{1.5}$  is due



others) however have contradicted the view that the incidence of emphysema is higher in patients with asthma. Gloor (1954) arrived at the opposite conclusion. This contradiction may be explained in the words of Jelp (1961) "Wir glauben, dass die Diskrepanz zwischen klinischen und histopathologischen Ergebnissen zum Teil auf einer unterschiedlichen Auslegung des Emphysembegriffes beruht. Viele Kliniker bezeichnen jede Lungenüberblähung zumal wenn sie längere Zeit besteht, als Emphysem während von pathologisch anatomischer Seite das Vorhandensein bestimmter morphologischer Merkmale für die Diagnose eines Emphysems im engeren Sinne gefordert wird"

An increased ratio of FRC/TLC and RV/TLC will be subsequently referred to in this discussion as "hyperinflation" and will be regarded as being consistent with but not specifically evidential of emphysema.

In comparison with the control material the asthma series as a whole showed on an average a significantly reduced ventilation capacity. Large variations were found in this series for each of the different tests. These variations, however, were observed not only for the material as a whole but also within the groups formed according to factors representing the estimated degree of severity of the disease, such as the number of sickness points, the duration etc. One possible reason is that the patients — in spite of the fact that in reality their asthma was of similar degrees of severity — exhibited considerable differences in ventilation capacity. This could be due to individ-

ual dissimilarities, for example the difference in the capacity of the lung tissue to resist strain of the type caused by bronchial constriction. The different errors of the method also play a role. The error in estimating the degree of severity of the disease, for example, is an important factor in the analysis of the results. The fact that an error of method exists is due not only to lack of memory and expressive ability on the part of some patients but also to the fact that different patients do not measure the degree of severity in the same way and experience their symptoms differently even when these are in principle of the same type and grade. Another factor of considerable importance was the difficulty in ascertaining that the subjective condition of a patient at the time of an investigation was optimal with respect to the asthma. These factors, together with others such as motivation, muscular strength, degree of training etc., contribute to the relatively wide ranges in the ventilation capacity and lung volumes even in groups of patients in whom the asthma was assessed as having been of a relatively similar degree of severity. This line of reasoning obviously holds in principle for the physical work capacity and hemodynamic functions also.

When the mean observed values for  $FEV_{1.0}$ ,  $FEV_x$ ,  $MVV_{40}$  and  $MVV_1$  were calculated separately for the asthma material as a whole a highly significant difference was found from the mean predicted values. For each of these tests significant differences were also shown between the different groups on classification of the material

according to the estimated degree of severity of the disease. These results thus support the view that there was some degree of relationship, as expected, between the estimated degree of severity and the ventilation capacity value found.

The degree of deviation between the observed and predicted values should be regarded with some caution, however, since there is no available control material that simultaneously fulfils all the criteria of "normal" material in the true sense of this word, which include the demand for a completely random selection of subjects from the total population.

It is probable that the differences found between the asthma series and the control material can be explained to a certain extent by the fact that the methods were not identical in practical (though identical in principle). The differences between the mean values for the two series were, however, of a considerable order of size, and it was also found that with regard to the variables for which the difference was greatest there was also a considerable difference between group A and group C, i.e. between the group that was estimated by the number of sickness points to have the greatest degree of severity since the onset of its asthma and the group with the lowest degree.

On adding the mean deviation from the predicted value for each of the test  $FEV_{1.5}$ ,  $FEV_{1.0}$ ,  $MVV_{1.5}$  and  $MVV_{1.0}$  for the whole main asthma series it was found that the deviation for the latter two was the greatest, i.e. about 45 percent units. The cor-

responding deviations for  $FEV_{1.5}$  and  $FEV_{1.0}$  were about 30 and 20 percent units respectively. This supports the view that a test of maximum ventilation capacity is to be preferred for an assessment of the reduction in ventilatory function in a material such as this. The possible advantage to the subject in being able to choose his own respiratory frequency did not appear to be greater for the patients in the asthma series than for the control persons. Regardless of whether a fixed frequency of 40 breaths per minute was used in the maximum ventilation capacity test or whether the patient himself chose the frequency the asthma patients on an average attained practically the same percentage of the predicted value. Thus for the asthma patients one of these two tests was probably adequate for assessing the influence of the disease on the ventilatory function. This assumption is supported by the fact that when a selection was made from the 118 asthma patients of firstly the 20 patients with the least favourable values of the  $MVV_{1.5}$  test, and secondly the 20 patients with the least favourable values at the  $MVV_{1.0}$  test, no fewer than 17 were common to each group. This indicated a highly significant relationship between these two tests of ventilation capacity.

The somewhat smaller mean difference from the control material shown by the  $FEV_{1.5}$  test, compared with the  $MVV$  tests, is probably connected, *inter alia* with the smaller degree of physical exertion associated with the performance of this test. The even smaller difference with regard to  $FEV_{1.0}$  is due

to the fact that the mean vital capacity showed some reduction in comparison with the control material

The four indices measuring ventilation capacity agree as regards the differences between the mean value for those patients who did not attain as many as 1000 total sickness points and that for the patients who attained a higher number

A regression analysis of the importance of certain case-history data to the results of different variables measuring respiratory function was performed. By this means a relationship was found between the intensity of the disease during the five-year period almost immediately preceding the investigation (1957-61) and the results of the actual function tests for the variables  $FEV_{1.0}$ ,  $FEV_{2.0}$ ,  $WV_{1.0}$  and  $WV_{2.0}$ . Five different interpretations may be suggested for this relationship

- 1 The intensity of the disease had a deteriorating effect on the ventilation capacity but this did not influence the intensity of the disease. With this interpretation the sickness points for 1957-61 constitute a genuine factor (a measure of the intensity) that would explain the ventilation capacity value found at the present study

- 2 The ventilation capacity had remained relatively stationary during a succession of years and had influenced the number of days of incapacity. In other words the intensity of the disease during 1957-61 was causally

dependent upon the ventilation capacity and not vice versa.

- 3 There was reciprocal effect between the intensity of the disease and the ventilation capacity for example the intensity of the disease during 1957 influenced the lung function state for the same year and this in turn influenced the intensity of the disease during 1958, and so on.
- 4 Both the intensity of the disease and the ventilation capacity were influenced in the same direction by other factors, e.g. certain forms of therapy. In other words both the disease intensity and the ventilation capacity were causally dependent on a common factor.
- 5 Some patients behaved according to point 1, others according to point 2 and still others according to point 3 and so on, or according to combinations of these alternatives.

The present study does not permit any conclusions, on the basis of purely statistical data as to which of these interpretations is correct. Some hypothetical reasoning is, however possible. The relationship between the intensity of the disease during the years previous to 1957 and the ventilation capacity at the present investigation was found on analysis to be negligible. If the relationship between the ventilation capacity at the beginning of the 1950's and that at the beginning of the 1960's were such that slow change (improvement or deterioration) of this function had occurred, alternative 1 could hardly be applicable. If it had applied here, the

partial correlation between the ventilation capacity at the present investigation and the intensity of the disease before 1957 — eliminating the disease intensity for the period 1957—61 — would clearly deviate from zero. However the partial correlation between the disease intensity before 1957 and the ventilation capacity at the present investigation — eliminating the influence of the disease intensity of 1957—61 — was weak, varying between  $-0.08$  and  $+0.04$  for the variables  $FEV_{10}$ ,  $FEV_{25}$ ,  $MVV_{50}$  and  $MVV_{75}$ . In order to determine whether the reduction in ventilation capacity is slowly reversible or can change markedly during a period of five years, a follow up study would be required. It is as yet too soon to perform such a study on the patients of the present series.

With regard to alternatives 2 and 3 a follow up study would be desirable from the point of view of obtaining a measure of the relationship between the ventilation capacity at two different time points with an interval of 5—10 years.

On the basis of clinical experience it may be said that a definite impression is obtained that in the short view the ventilation capacity is dependent on the intensity of the disease. As to the long term relationship it does not seem possible to express an opinion with certainty in cases of uncomplicated bronchial asthma.

With regard to alternative 4 there appeared to be no common factors that could have had appreciable influence on the ventilation capacity and the disease intensity simultaneously in the same favourable direction. On the

other hand there were factors that probably influenced the intensity of the disease by preventing or counteracting the occurrence of for example, asthmatic attacks (e.g. corticosteroids). Other factors of the type that may influence the ventilation capacity as such probably also played some role (e.g. breathing exercises). If both of these forms of therapy occur at the same time they then acquire the character of a common factor. During the period 1957—61 it was only in exceptional cases among these patients that therapy of both these types was given, and therefore alternative 4 does not appear to be worthy of appreciable consideration.

On regression analysis a relationship was found between the over all degree of severity of the disease and the ratio of the residual volume in particular but also the functional residual capacity to the total lung capacity. This relationship did not appear to depend primarily on the intensity of the disease during the years almost immediately preceding the time of the investigation. On analysis the relationship between these ratios and the intensity of the disease during the years mentioned was found to be relatively small. The reversibility factor appeared to be greater with regard to the effect of the disease on the ventilation capacity — as expressed by the function tests  $FEV_{10}$ ,  $FEV_{25}$ ,  $MVV_{50}$  and  $MVV_{75}$  — than with regard to the effect on certain lung volume relations — as expressed by the ratios of not only the residual volume but also the functional residual capacity to the total lung capacity.

The *physical work capacity* of the patients in the asthma material was determined in order to obtain a quantitative assessment of the respiratory and circulatory functional impairment in particular directly or indirectly caused by the disease.

Work tests as methods of clinical investigation in pulmonary diseases have been used previously, among others by Dahlstrom (1905 and 1957) on patients with pulmonary tuberculosis and by Svanborg (1961) on patients with pulmonary sarcoidosis. Both authors found on an average a low physical work capacity in the sitting position.

It was considered of especial interest in the present investigation to study whether the estimated degree of severity of the disease had any relationship — and if so to what extent — with the physical work capacity as observed in these tests. It was also of great interest to determine the degree of influence of a number of given factors in the case history of the asthma patient on the work capacity as estimated by the function variables  $W_{100}$  and  $W_{max}$ . The ranges of values for the physical work capacity in the asthma material may be regarded as an expression, among other things, of constitution and the effects of the disease. In comparing the asthma patients with the persons in the control material the two function variables  $W_{100}$  and  $W_{max}$  were systematically studied. For these two variables — in contrast to  $W_{100}$ , for example — values were obtained for almost all patients. It was of particular importance on comparing different groups within the asthma series

to be able to base these comparisons on function variables for which a value had been obtained for the majority of the patients.

The mean observed value for the physical work capacity of the patients in the asthma series differed significantly from the mean predicted value. A conceivable reason for this difference was the change in the way of life, from a physical aspect, which the disease appeared to have brought about in the majority of these patients. This assumption was strongly supported by the knowledge gained about these patients on the many occasions of interview and often detailed conversation. This change in their way of living which to no small extent was characterized by the avoidance of physical exertion, was due to many different factors. One such factor was that during periods of symptoms the asthma rendered work difficult or impossible, even when only moderate physical strain was demanded. Another was that during so-called free or almost free intervals, physical strain provoked asthma in several of these patients.

In the asthma series a relationship was found between working capacity and ventilation capacity. A possible causative chain of events leading to this relationship may be that the disease primarily gives a reduction of ventilation capacity which contributes a sedentary way of living and this in turn may result in a low degree of physical exercise and a reduced working capacity.

As in the case of the ventilation

capacity the physical work capacity as observed in the present study appeared to have some relationship with the estimated degree of severity of the disease. For the mean values of both  $W_{120}$  and  $W_{max}$  there was a significant difference between those patients who since the onset of the disease had attained at least 2000 sickness points and those who had fewer than 1000 points. A relatively large number of case-history factors studied appeared to influence the variable  $W_{max}$  to such an extent that on classification of the patients according to sub-groups of these factors a significant difference was found between the groups as regards this variable. Some case history factors also seemed to be of similar importance to the variable  $W_{120}$ , but these were fewer in number than for  $W_{max}$ .

A significant relationship was found for the patients in the asthma series between ventilation capacity ( $FEV_{1.0}$ ,  $FEV_{1.5}$ ,  $MEV_{1.0}$  and  $MEV_{1.5}$ ) and work capacity ( $W_{120}$  and  $W_{max}$ ). The coefficient of correlation was about 0.30 (\*\*). The partial correlation between  $W_{120}$  and the total number of sickness points — when the influence of the ventilation capacity had been eliminated — was about 0.03, i.e. low. This appears to indicate that the influence of the disease measured by sickness points, on the physical work capacity is exerted to the greatest part via the influence on the ventilation capacity.

It has been assumed — which is reasonable from a clinical aspect — that the ventilation capacity is of some significance to the physical work capa-

city. Whether — and if so to what extent — the physical work capacity has any influence on the ventilation capacity cannot be stated with certainty. It seems a reasonable supposition, however, that a low degree of physical exercise resulting in a relatively small muscle mass may have some influence — although probably small — on the ventilation capacity.

In normal adults there is no definite correlation between physical work capacity determined by the submaximal work test, and vital capacity (Wahlund, 1948, P. O. Åstrand, 1957), ventilation capacity (P. O. Åstrand, 1952, Irma Åstrand, 1960, Grimby and Söderholm, 1963) or the ratio of the residual volume to the total lung capacity (Wahlund, 1948). Dahlström (1937) showed in patients with pulmonary tuberculosis that there was a statistically significant correlation between physical work capacity and both vital capacity and maximal ventilation capacity. No significant relationship was shown, on the other hand, between physical work capacity and the ratio of residual volume to total lung capacity.

It was considered of interest to investigate the conditions in the present series of patients. As in Dahlström's (1937) material, there appeared to be a statistically significant correlation between physical work capacity and both vital capacity and ventilation capacity. As regards the latter the relationship was significant in the asthma series both with the forced expiratory volume per one second and with the maximal ventilation capacity. The relationship between physical work

The physical work capacity of the patients in the asthma material was determined in order to obtain a quantitative assessment of the respiratory and circulatory functional impairment in particular directly or indirectly caused by the disease.

Work tests as methods of clinical investigation in pulmonary diseases have been used previously among others by Dahlistrom (1955 and 1957) on patients with pulmonary tuberculosis, and by Svanborg (1961) on patients with pulmonary sarcoidosis. Both authors found on an average a low physical work capacity in the sitting position.

It was considered of especial interest in the present investigation to study whether the estimated degree of severity of the disease had any relationship — and if so to what extent — with the physical work capacity as observed in these tests. It was also of great interest to determine the degree of influence of a number of given factors in the case history of the asthma patient on the work capacity as estimated by the function variables  $W_{120}$  and  $W_{max}$ . The ranges of values for the physical work capacity in the asthma material may be regarded as an expression among other things, of constitution and the effects of the disease. In comparing the asthma patients with the persons in the control material the two function variables  $W_{120}$  and  $W_{max}$  were systematically studied. For these two variables — in contrast to  $W_{120}$ , for example — values were obtained for almost all patients. It was of particular importance on comparing different groups within the asthma series

to be able to base these comparisons on function variables for which a value had been obtained for the majority of the patients.

The mean observed value for the physical work capacity of the patients in the asthma series differed significantly from the mean predicted value. A conceivable reason for this difference was the change in the way of life from a physical aspect which the disease appeared to have brought about in the majority of these patients. This assumption was strongly supported by the knowledge gained about these patients on the many occasions of interview and often detailed conversation. This change in their way of living, which to no small extent was characterized by the avoidance of physical exertion was due to many different factors. One such factor was that during periods of symptoms the asthma rendered work difficult or impossible even when only moderate physical strain was demanded. Another was that during so-called free or almost free intervals, physical strain provoked asthma in several of these patients.

In the asthma series a relationship was found between working capacity and ventilation capacity. A possible causative chain of events leading to this relationship may be that the disease primarily gives a reduction of ventilation capacity which contributes a sedentary way of living and this in turn may result in a low degree of physical exercise and a reduced working capacity.

As in the case of the ventilation

capacity the physical work capacity as observed in the present study appeared to have some relationship with the estimated degree of severity of the disease. For the mean values of both  $W_{120}$  and  $W_{max}$  there was a significant difference between those patients who since the onset of the disease had attained at least 2000 sickness points and those who had fewer than 1000 points. A relatively large number of case-history factors studied appeared to influence the variable  $W_{max}$  to such an extent that on classification of the patients according to sub-groups of these factors a significant difference was found between the groups as regards this variable. Some case-history factors also seemed to be of similar importance to the variable  $W_{120}$ , but these were fewer in number than for  $W_{max}$ .

A significant relationship was found for the patients in the asthma series between ventilation capacity ( $FEV_{1.0}$ ,  $FEV_{1.5}$ ,  $SVV_{0.5}$  and  $SVV_{1.0}$ ) and work capacity ( $W_{120}$  and  $W_{max}$ ). The coefficient of correlation was about 0.30 ( ). The partial correlation between  $W_{120}$  and the total number of sickness points — when the influence of the ventilation capacity had been eliminated — was about 0.05, i.e. low. This appears to indicate that the influence of the disease measured by sickness points, on the physical work capacity is exerted to the greatest part via the influence on the ventilation capacity.

It has been assumed — which is reasonable from a clinical aspect — that the ventilation capacity is of some significance to the physical work capacity.

Whether — and if so to what extent — the physical work capacity has any influence on the ventilation capacity cannot be stated with certainty. It seems a reasonable supposition, however, that a low degree of physical exercise resulting in a relatively small muscle mass may have some influence — although probably small — on the ventilation capacity.

In normal adults there is no definite correlation between physical work capacity determined by the submaximal work test, and vital capacity (Wahlund, 1948, P. O. Åstrand, 1952) ventilation capacity (P. O. Åstrand, 1952, Irma Åstrand, 1960 Grimby and Söderholm, 1963) or the ratio of the residual volume to the total lung capacity (Wahlund, 1948). Dahlström (1957) showed in patients with pulmonary tuberculosis that there was a statistically significant correlation between physical work capacity and both vital capacity and maximal ventilation capacity. No significant relationship was shown, on the other hand, between physical work capacity and the ratio of residual volume to total lung capacity.

It was considered of interest to investigate the conditions in the present series of patients. As in Dahlström's (1957) material, there appeared to be a statistically significant correlation between physical work capacity and both vital capacity and ventilation capacity. As regards the latter the relationship was significant in the asthma series both with the forced expiratory volume per one second and with the maximal ventilation capacity. The relationship between physical work



The *physical work capacity* of the patients in the asthma material was determined in order to obtain a quantitative assessment of the respiratory and circulatory functional impairment in particular directly or indirectly caused by the disease.

Work tests as methods of clinical investigation in pulmonary diseases have been used previously among others by Dahlström (1955 and 1957) on patients with pulmonary tuberculosis and by Svanborg (1961) on patients with pulmonary sarcoidosis. Both authors found on an average a low physical work capacity in the sitting position.

It was considered of especial interest in the present investigation to study whether the estimated degree of severity of the disease had any relationship — and if so to what extent — with the physical work capacity as observed in these tests. It was also of great interest to determine the degree of influence of a number of given factors in the case history of the asthma patient on the work capacity as estimated by the function variables  $W_{120}$  and  $W_{max}$ . The ranges of values for the physical work capacity in the asthma material may be regarded as an expression, among other things of constitution and the effects of the disease. In comparing the asthma patients with the persons in the control material the two function variables  $W_{120}$  and  $W_{max}$  were systematically studied. For these two variables — in contrast to  $W_{120}$ , for example — values were obtained for almost all patients. It was of particular importance on comparing different groups within the asthma series

to be able to base these comparisons on function variables for which a value had been obtained for the majority of the patients.

The mean observed value for the physical work capacity of the patients in the asthma series differed significantly from the mean predicted value. A conceivable reason for this difference was the change in the way of life, from a physical aspect which the disease appeared to have brought about in the majority of these patients. This assumption was strongly supported by the knowledge gained about these patients on the many occasions of interview and often detailed conversation. This change in their way of living which to no small extent was characterized by the avoidance of physical exertion, was due to many different factors. One such factor was that during periods of symptoms the asthma rendered work difficult or impossible, even when only moderate physical strain was demanded. Another was that during so-called free or almost free intervals, physical strain provoked asthma in several of these patients.

In the asthma series a relationship was found between working capacity and ventilation capacity. A possible causative chain of events leading to this relationship may be that the disease primarily gives a reduction of ventilation capacity which contributes to a sedentary way of living and this in turn may result in a low degree of physical exercise and a reduced working capacity.

As in the case of the ventilation

capacity the physical work capacity as observed in the present study appeared to have some relationship with the estimated degree of severity of the disease. For the mean values of both  $W_{120}$  and  $W_{max}$  there was a significant difference between those patients who since the onset of the disease had attained at least 2000 sickness points and those who had fewer than 1000 points. A relatively large number of case-history factors studied appeared to influence the variable  $W_{max}$  to such an extent that on classification of the patients according to sub-groups of these factors a significant difference was found between the groups as regards this variable. Some case-history factors also seemed to be of similar importance to the variable  $W_{120}$ , but these were fewer in number than for  $W_{max}$ .

A significant relationship was found for the patients in the asthma series between ventilation capacity ( $FEV_1$ ,  $FEV_2$ ,  $MMV_{60}$  and  $MMV_1$ ) and work capacity ( $W_{120}$  and  $W_{max}$ ). The coefficient of correlation was about 0.30. The partial correlation between  $W_{120}$  and the total number of sickness points — when the influence of the ventilation capacity had been eliminated — was about 0.05, i.e. low. This appears to indicate that the influence of the disease measured by sickness points, on the physical work capacity is exerted to the greatest part via the influence on the ventilation capacity.

It has been assumed — which is reasonable from a clinical aspect — that the ventilation capacity is of some significance to the physical work capa-

city. Whether — and if so to what extent — the physical work capacity has any influence on the ventilation capacity cannot be stated with certainty. It seems a reasonable supposition, however, that a low degree of physical exercise resulting in a relatively small muscle mass may have some influence — although probably small — on the ventilation capacity.

In normal adults there is no definite correlation between physical work capacity determined by the submaximal work test, and vital capacity (Wahlund, 1948, P. O. Åstrand, 1952) ventilation capacity (P. O. Åstrand, 1952 Irma Åstrand, 1960 Grimby and Söderholm, 1963) or the ratio of the residual volume to the total lung capacity (Wahlund, 1948). Dahlström (1957) showed in patients with pulmonary tuberculosis that there was a statistically significant correlation between physical work capacity and both vital capacity and maximal ventilation capacity. No significant relationship was shown, on the other hand, between physical work capacity and the ratio of residual volume to total lung capacity.

It was considered of interest to investigate the conditions in the present series of patients. As in Dahlström's (1957) material, there appeared to be a statistically significant correlation between physical work capacity and both vital capacity and ventilation capacity. As regards the latter the relationship was significant in the asthma series both with the forced expiratory volume per one second and with the maximal ventilation capacity. The relationship between physical work

capacity and the ratio of residual volume to total lung capacity on the other hand appeared to be negligible this was also in agreement with Dahlström's findings in tuberculous patients

In the asthma series however the ratio  $RV/TLC$  showed on an average, a relatively large deviation from the predicted value, in an unfavourable direction. It would not have been unexpected if this variable had been found to be of significant importance to the physical work capacity in the present series of patients

Large variations in the degree of severity of the asthma were also found in the patients studied with regard to possible secondary effects of the disease on cardiac function and pulmonary circulation. As in the study of the respiratory function and physical work capacity the patients were divided into groups according to the estimated degree of severity of the disease. The findings in the hemodynamic investigations were compared in the different groups. Mutual comparisons such as these were of especial value since a sufficiently large control material extending over the whole age range of 30—60 years was not available

The blood pressures in the pulmonary artery and in the lung capillaries were normal during a resting state in all patients studied. The flow resistance of the pulmonary vessels (as measured by  $\Delta$  units i.e. the pressure gradient in mm Hg per blood flow in litres/min) showed a value of less than 2 during the resting state for all patients with the exception of five men and seven women in whom the value ex-

ceeded 2 but did not reach 3 units. No pulmonary hypertension or noteworthy increase of the pulmonary vascular resistance need thus necessarily occur during a resting state even in patients with prolonged and severe bronchial asthma. The disease in this series of patients therefore differed, as regards the effect on the pulmonary circulation from the chronic obstructive pulmonary diseases studied in the series of patients from other countries which were found to have a tendency to pulmonary hypertension. One possible explanation for this difference is that the patients in the present investigation represent the course in uncomplicated bronchial asthma while in the other series the conditions were affected by a varying collection of such factors as chronic infection, air pollution and sometimes cigarette smoking. It seems reasonable therefore to draw the conclusion that even prolonged and relatively severe bronchial asthma does not as a rule lead to pulmonary hypertension at rest if it is not complicated by other conditions giving rise to lung tissue destruction. The series of patients reported here showed a tendency to an increase in the ratio of the residual volume to the total lung capacity but the absence of pulmonary hypertension appears to indicate that this was not due to extensive destructive pulmonary emphysema but only to a less complicated state of hyperinflation

The change in the pulmonary arterial pressure during physical exercise on the other hand exhibited a relationship with the estimated degree of severity of the disease — measured by

sickness points — in this group of patients. This relationship was statistically significant for the intensity of the disease during the five years almost immediately preceding the time of the investigation. If on the contrary the over all degree of severity was taken into account, no significant tendency to a higher rise in pressure on increase in the number of sickness points was found. This supports the view that bronchial asthma that has been intense for a few recent years does influence the pulmonary circulation even during a symptom free state, at least during physical exercise as shown by rise in pressure this has not been shown during a resting state. It also may indicate that the deterioration is reversible i.e. it subsides when the disease becomes less active.

The effect of anoxic anoxia on the pulmonary circulation in man has been studied in normal persons by several authors including Motley, Coorndam, Werk, Himmelstein and Drendale (1917), Westcott, Fowler Scott, Hansen and McGuire (1931), Asmusen and Nielsen (1934), Boll, Solent and Thier (1937), Fritts, Harris, Claus (1941) and Coorndam (1958) and Fishman, Fritts and Coorndam (1960) and also a few investigations on patients with chronic pulmonary disease (Dexter, Wittenberger, Gorlin, Lewis, Haynes and Spiegel 1951 and Fishman, Votement, Himmelstein and Coorndam 1954). Of patients with chronic pulmonary diseases about 1/3 appear to react to slight anoxic anoxia (about 10% in  $F_{O_2}$ ) with a significant increase in the pulmonary arterial pressure while the cardiac output is unchanged

or somewhat increased (Fishman et al 1952). More pronounced anoxic anoxia i.e. leading to arterial oxygen saturation of less than 80% gives rise, however in the majority of patients with pulmonary emphysema to a considerable increase in the cardiac minute volume (Wade and Bishop 1962). For normal subjects Fritts et al. (1948) and Fishman et al. (1960) reported that during the breathing of 12% oxygen the cardiac output under steady state conditions increased by about 20% in comparison with the normal resting conditions. No studies of the effect of anoxic anoxia on patients with bronchial asthma of the type present in the patients reported here appear however to have been made. In the present investigation the patients were submitted to fairly marked anoxic anoxia (11%  $F_{O_2}$ , mean arterial oxygen saturation of 70%). The pulmonary arterial pressure thereby rose significantly but only relatively moderately, no significant change in the cardiac output was shown. Neither was any noteworthy difference observed between groups in which the asthma had shown different degrees of severity. Two patients who during anoxic anoxia developed transient but considerable pulmonary hypertension did not show any deviation from the general tendency in the rest of the material towards an insignificant influence on the cardiac output by the anoxia. It is true that the pressure in the lung capillaries was not recorded during the anoxic anoxia, and therefore the possibility of a small increase in the pulmonary capillary pressure cannot be excluded. Assuming this

capacity and the ratio of residual volume to total lung capacity on the other hand appeared to be negligible this was also in agreement with Dahlström's findings in tuberculous patients

In the asthma series, however the ratio RV/TLC showed on an average a relatively large deviation from the predicted value in an unfavourable direction. It would not have been unexpected if this variable had been found to be of significant importance to the physical work capacity in the present series of patients

Large variations in the degree of severity of the asthma were also found in the patients studied with regard to possible secondary effects of the disease on cardiac function and pulmonary circulation. As in the study of the respiratory function and physical work capacity the patients were divided into groups according to the estimated degree of severity of the disease. The findings in the hemodynamic investigations were compared in the different groups. Mutual comparisons such as these were of especial value since a sufficiently large control material extending over the whole age range of 30—60 years was not available.

The blood pressures in the pulmonary artery and in the lung capillaries were normal during a resting state in all patients studied. The flow resistance of the pulmonary vessels (as measured by R units, i.e. the pressure gradient in mm Hg per blood flow in litres/min) showed a value of less than 2 during the resting state for all patients with the exception of five men and seven women in whom the value ex-

ceeded 2 but did not reach 3 units. No pulmonary hypertension or noteworthy increase of the pulmonary vascular resistance need thus necessarily occur during a resting state even in patients with prolonged and severe bronchial asthma. The disease in this series of patients therefore differed as regards the effect on the pulmonary circulation from the chronic obstructive pulmonary diseases studied in the series of patients from other countries which were found to have a tendency to pulmonary hypertension. One possible explanation for this difference is that the patients in the present investigation represent the course in uncomplicated bronchial asthma, while in the other series the conditions were affected by a varying collection of such factors as chronic infection, air pollution and sometimes cigarette smoking. It seems reasonable therefore to draw the conclusion that even prolonged and relatively severe bronchial asthma does not as a rule lead to pulmonary hypertension at rest if it is not complicated by other conditions giving rise to lung tissue destruction. The series of patients reported here showed a tendency to an increase in the ratio of the residual volume to the total lung capacity but the absence of pulmonary hypertension appears to indicate that this was not due to extensive destructive pulmonary emphysema but only to a less complicated state of hyperinflation.

The change in the pulmonary arterial pressure during physical exercise, on the other hand, exhibited a relationship with the estimated degree of severity of the disease — measured by

sickness points — in this group of patients. This relationship was statistically significant for the intensity of the disease during the five years almost immediately preceding the time of the investigation. If on the contrary the over-all degree of severity was taken into account, no significant tendency to a higher rise in pressure on increase in the number of sickness points was found. This supports the view that bronchial asthma that has been intensive for a few recent years does influence the pulmonary circulation even during a symptom free state, at least during physical exercise, as shown by rise in pressure this has not been shown during a resting state. It also may indicate that the deterioration is reversible, i.e. it subsides when the disease becomes less active.

The effect of anoxic anoxia on the pulmonary circulation in man has been studied in normal persons by several authors, including Motley Courmand, Werko Himmelstein and Drendale (1947) Westcott, Fowler Scott Hauenstein and McGuire (1951) Asmusen and Nielsen (1956) Bolt, Valentin and Tietz (1957) Fritts, Harris, Clams, Odell and Courmand (1958) and Fishman and Fritts and Courmand (1960) and also in a few investigations on patients with chronic pulmonary disease (Dexter Whittenberger Gorlin, Lewis, Haynes and Spiegl, 1951 and Fishman, McClement, Himmelstein and Courmand, 1952). Of patients with chronic pulmonary diseases, about 1/3 appear to react to slight anoxic anoxia (about 18% in  $\text{FIO}_2$ ) with a significant increase in the pulmonary arterial pressure while the cardiac output is unchanged

or somewhat increased (Fishman et al 1952). More pronounced anoxic anoxia, i.e. leading to arterial oxygen saturation of less than 80%, gives rise, however in the majority of patients with pulmonary emphysema to a considerable increase in the cardiac minute volume (Wade and Bishop 1962). For normal subjects, Fritts et al. (1958) and Fishman et al. (1960) reported that during the breathing of 12% oxygen the cardiac output under steady state conditions increased by about 20% in comparison with the normal resting conditions. No studies of the effect of anoxic anoxia on patients with bronchial asthma of the type present in the patients reported here appear however to have been made. In the present investigations the patients were submitted to fairly marked anoxic anoxia (11%  $\text{FIO}_2$ , mean arterial oxygen saturation of 76%). The pulmonary arterial pressure thereby rose significantly but only relatively moderately — no significant change in the cardiac output was shown. Neither was any noteworthy difference observed between groups in which the asthma had shown different degrees of severity. Two patients who during anoxic anoxia developed transient but considerable pulmonary hypertension did not show any deviation from the general tendency in the rest of the material towards an insignificant influence on the cardiac output by the anoxia. It is true that the pressure in the lung capillaries was not recorded during the anoxic anoxia, and therefore the possibility of a small increase in the pulmonary capillary pressure cannot be precluded. Assuming this



explained by the lower oxygen uptake capacity of the asthma patients (partly due to the difference in sex proportion) and does not necessarily indicate an abnormal change of circulatory function. The average arterio-venous oxygen difference per heart rate was on the other hand approximately the same for the asthma patients and the control material, although the individual variation was rather high. The asthma patients may therefore from this point of view be regarded as having a "normokinetic" circulation. During muscular work, the cardiac output in relation to oxygen uptake was also found to increase similarly in the asthma patients and in the normal groups. At rest, however the cardiac output per oxygen uptake was relatively low in the asthma patients this may possibly depend on the difference in age (Granath, Jonsson and Strandell, 1961) the asthma patients being older than the control materials. For comparison Holmgren, Jonsson, Linderholm, Sjöstrand and Ström (1958) and Donald, Bishop and Wade (1954) found that a proportion of patients with mitral stenosis, in sinus rhythm were "hypokinetic" i.e. they exhibited both low cardiac minute volume in relation to the oxygen uptake and a high arterio-venous oxygen difference in relation to the heart rate. On the other hand, the opposite condition, i.e. a hyperkinetic circulation, held for persons with so-called vasoregulatory stenosis (Holmgren et al., 1957). These individuals thus showed a high cardiac minute volume but a low peripheral utilization of the oxygen content.

In a group of older normal men, Granath et al. (1961) found a mean arterio-venous oxygen difference in relation to oxygen uptake that was in closer agreement with the mean value for the asthma series. The significance of age is appreciable by the fact that regardless of the estimated degree of severity of the disease the patients in the asthma series with a higher mean age had a somewhat higher  $a-v O_2$  difference in relation to oxygen uptake than the patients with a lower mean age. Increasing age is also accompanied by a decrease of the maximal heart rate level and this change may be accompanied by a tendency to increased  $a-v O_2$  difference in relation to heart rate.

For each of the function variables, such as for example the pressure in the pulmonary artery the group of patients showing the largest deviation from the normal values were selected. Pairs of variables were then studied to find out how many patients were common to the two groups. It was found that for the mean pressure in the pulmonary artery during anoxic anoxia, and the arterial saturation also during anoxic anoxia, a larger number of patients than predicted were common to the two groups. The difference between the observed and predicted value was significant. A similar significant difference was also found for both combinations, when the mean pressure in the pulmonary artery during exercise was paired firstly with the mean pressure in the pulmonary artery during anoxic anoxia and secondly with the pulmonary vascular resistance during



pressure to be unchanged, the mean pulmonary vascular resistance for the series as a whole is then higher during the anoxia than during a normal resting state. This increase in resistance may be interpreted as being due to alveolar hypoxia.

The physical work capacity was found on the whole to be the same in the supine as in the sitting position which is in agreement with the findings of Holmgren, Jonsson and Sjöstrand (1960) in normal subjects. Patients with pulmonary sarcoidosis (Svanborg 1961) on the other hand like orthostatic patients, exhibited a higher capacity in the supine than in the sitting position. The fact that in the asthma series the heart rate at certain loads was practically the same in the supine and the sitting position, and that the calculated mean work load in the supine and sitting positions at heart rates of 130 and 150 was approximately the same, supports the view that the orthostatic effect in the sitting position was not of any noteworthy order of size in these patients. In the orthostatic test also the mean increase in heart rate was found to be of a normal order of size.

For the stroke volume at rest no notable difference was found in the present material on classification into groups according to the estimated degree of severity of the disease. During exercise at 300 lpm/min for men and 200 lpm/min for women, the stroke volume increased in comparison with the resting conditions by an average of 26 % for the former and 16 % for the latter. The increase was more marked than that reported previously

for normal persons (Bevegård, Holmgren and Jonsson, 1960 and Holmgren et al 1960) but the latter persons were on an average younger. The tendency in the asthma patients to a relatively large increase in the stroke volume on changing over from a resting state to exercise is more similar to the results published in a preliminary report by Grannath, Jonsson and Sirandell (1961). These results were based on a material of normal men of ages 60—83 years. The differences between the asthma series and the control materials lie essentially in the resting values. The asthma material had a lower stroke volume at rest both absolute and in relation to body size. This was possibly due to an inadequate distribution of the blood volume. When exercise was commenced redistribution probably took place, with the result that both the central blood volume and the stroke volume increased. One factor possibly contributing to the significantly increased stroke volume during the first work load was an increase in myocardial "contractility" in effect such as this could not be precluded in the asthma series.

It is of interest to analyse the "circulatory efficiency" of the asthma patients, i.e. cardiac output in relation to oxygen need and heart rate. The patients in the present series showed on an average a higher arterio-venous oxygen difference in relation to oxygen uptake than that reported for a group of normal individuals (most of whom were males) (Holmgren, Jonsson, Levander, Linderholm, Sjöstrand and Ström 1957; Holmgren et al. 1960; Bevegård et al 1960). This may be

explained by the lower oxygen uptake capacity of the asthma patients (partly due to the difference in sex proportion) and does not necessarily indicate an abnormal change of circulatory function. The average arterio-venous oxygen difference per heart rate was on the other hand approximately the same for the asthma patients and the control material, although the individual variation was rather high. The asthma patients may therefore from this point of view be regarded as having a "normokinetic" circulation. During muscular work, the cardiac output in relation to oxygen uptake was also found to increase similarly in the asthma patients and in the normal groups. At rest, however the cardiac output per oxygen uptake was relatively low in the asthma patients; this may possibly depend on the difference in age (Granath, Jonason and Strandell, 1961) the asthma patients being older than the control materials. For comparison H. Ingren, Jonason, Linderholm, Sjöstrand and Sjöström, (1958) and Donald, Bishop and Wade (1954) found that a proportion of patients with mitral stenosis, in sinus rhythm, were "hypokinetic" i.e. they exhibited both a low cardiac minute volume in relation to the oxygen uptake and a high arterio-venous oxygen difference in relation to the heart rate. On the other hand, the opposite condition, i.e. a "hyperkinetic" circulation, held for persons with so-called vasoregulatory asthma (Holmgren et al. 1937). These individuals thus showed a high cardiac minute volume but a low peripheral utilization of the oxygen content.

In a group of older normal men, Granath et al. (1961) found a mean arterio-venous oxygen difference in relation to oxygen uptake that was in closer agreement with the mean value for the asthma series. The significance of age is appreciable by the fact that regardless of the estimated degree of severity of the disease the patients in the asthma series with a higher mean age had a somewhat higher a-v  $O_2$  difference in relation to oxygen uptake than the patients with a lower mean age. Increasing age is also accompanied by a decrease of the maximal heart rate level, and this change may be accompanied by a tendency to increased a-v  $O_2$  difference in relation to heart rate.

For each of the function variables, such as for example the pressure in the pulmonary artery the group of patients showing the largest deviation from the normal values were selected. Pairs of variables were then studied to find out how many patients were common to the two groups. It was found that for the mean pressure in the pulmonary artery during anoxic anoxia and the arterial saturation also during anoxic anoxia, a larger number of patients than predicted were common to the two groups. The difference between the observed and predicted value was significant. A similar significant difference was also found for both combinations, when the mean pressure in the pulmonary artery during exercise was paired firstly with the mean pressure in the pulmonary artery during anoxic anoxia and secondly with the pulmonary vascular resistance during

exercise. For practically all combinations a higher number of patients common to the two variables was observed than predicted, even if the difference was not significant. The variable that showed the lowest mean correlation with the other variables was  $RV/TLC$ , and that showing the next lowest was  $FEV_{1.0}$ . In consideration of the comparatively high increase in the ratio of the residual volume to the total lung capacity shown on an average by these patients, a greater degree of correlation between this variable and the rest might possibly have been expected.

Hurtado, Kaltreider, Fray, Brooks and McCann (1934) and Borden, Wilson, Fbert and Wells (1950) maintained that an increased  $RV/TLC$  ratio was an index of the degree of severity of emphysema. Herachfus, Bresnick and Segal (1953) found that according to this index all the patients in their material exhibited pronounced emphysema. It was pointed out however that to judge by the arterial oxygen saturation for example which among other factors was regarded by Baldwin et al. (1949) as particularly significant in this respect the patients only had moderate emphysema. In the present series the relationship between a high  $RV/TLC$  ratio and the pulmonary vascular resistance during exercise was not significant. This supported the view that in the patients in this series the degree of hyperinflation complicated by alveolar tissue destruction was only small or moderate. This is presuming that a relationship exists between pulmonary vascular resistance and emphysema — if by emphysema is

meant a condition of inflation complicated by alveolar wall destruction.

The hemodynamic findings in the patients in this asthma series were on the whole only slightly abnormal. This applied especially to the material as a whole, but also to the group of patients who according to the case history had been most severely affected. It was found also that it was not always in the patients who had had especially severe symptoms that the values showed the greatest difference from the normal values. It was therefore often of interest to make a separate study of those patients in whom this difference was found to be greatest. This group however exhibited a status that in many respects could be regarded as remarkably good in consideration of the fact that this material was composed of persons who during often large parts of their lives had been completely or partly disabled by asthma symptoms. It therefore seemed reasonable to attribute the relatively small extent of the abnormal deviations in the hemodynamic findings to the fact that the dilatation of the terminal bronchioles indicated by the increased residual volume was not followed by destruction in the bronchial walls of such a degree as to cause irreversible damage. The reason that in these asthma patients, in spite of the often greatly increased residual volume there did not appear to be any great functional consequences of the disease was assumed to be that the material did not include patients in whom the asthma was complicated by chronic bronchitis. It is considered probable that when bronchial asthma is com-

pliated by chronic bronchitis there is a great increase in the risk of emphysema as defined by the American Thoracic Society (1962) "Emphysema is an anatomical alteration of the lung characterized by an abnormal enlargement of the air spaces distal to the terminal, nonrespiratory bronchioles, accompanied by destructive changes of the alveolar walls" or the similar definition earlier proposed by the World Health Organization Committee on Chronic Cor Pulmonale (1961) Since it was considered essential to emphasize the difference between alveolar dilatation and "alveolar wall destruction" the former definitions were preferred — like Fletcher, Hugh Jones, McNicol and Pride (1963) did — to that given by the Ciba Foundation Symposium (1959) "Emphysema is a condition of the lung characterized by increase beyond the normal in the size of air spaces distal to the terminal bronchioles either from dilatation or from destruction of their walls"

Fletcher et al. (1963) made this distinction because alveolar dilatation was regarded as reversible, in contrast to irreversible destructive changes. The present investigations, however, have offered some support for the view that in patients with bronchial asthma hyperinflation does not appear to be characterized by reversibility to any great extent, but that this condition is probably less complicated than that present in emphysema. It is therefore from this point of view considered reasonable to distinguish between hyperinflation and emphysema in a material such as the present one also.

An attempt was made to form an

idea of the influence exerted on the hemodynamic functions by different factors representing the estimated degree of severity of the asthma. The values for each of certain different functions, e.g. the pressure in the pulmonary artery were studied in subgroups of each of these different factors, e.g. the number of sickness points. To obtain the best contrast two extreme groups were selected: the first comprised patients who showed the greatest difference from the normal values in a pathological direction, and the second in the opposite direction. Each extreme group comprised 20 patients (i.e. 36 per cent of the whole series). It was then found that for several of the functions the selected group with the pathological deviation had a significantly higher age than the other group. The former group as most of the older patients, also often showed a relatively high age at onset of the disease. It seems possible but cannot be stated with certainty that age had a deterring influence on the functional sequelae as regards the pulmonary circulation in this series of patients. On analysing the significance of different case history factors for some indices of ventilation capacity and lung volumes, it was found that age was on an average of greater influence in this respect among the asthma patients than among the control persons. This offers some support for the view that the consequences of bronchial asthma are of greater functional significance in older than in younger persons. The resistance of the tissues to the strain produced by asthma probably decreases with in-

exercise. For practically all combinations a higher number of patients common to the two variables was observed than predicted even if the difference was not significant. The variable that showed the lowest mean correlation with the other variables was  $RV/TLC$ , and that showing the next lowest was  $FEV_{1.0}$ . In consideration of the comparatively high increase in the ratio of the residual volume to the total lung capacity shown on an average by these patients a greater degree of correlation between this variable and the rest might possibly have been expected.

Hurtado, Kaltreider, Fray, Brooks and McCann (1934) and Borden, Wilson, Ebert and Wells (1950) maintained that an increased  $RV/TLC$  ratio was an index of the degree of severity of emphysema. Herschfus, Bresnick and Segal (1953) found that according to this index all the patients in their material exhibited pronounced emphysema. It was pointed out, however, that to judge by the arterial oxygen saturation, for example, which among other factors was regarded by Baldwin et al (1949) as particularly significant in this respect the patients only had moderate emphysema. In the present series the relationship between a high  $RV/TLC$  ratio and the pulmonary vascular resistance during exercise was not significant. This supported the view that in the patients in this series the degree of hyperinflation complicated by alveolar tissue destruction was only small or moderate. This is presuming that a relationship exists between pulmonary vascular resistance and emphysema — if by emphysema is

meant a condition of inflation complicated by alveolar wall destruction.

The hemodynamic findings in the patients in this asthma series were on the whole only slightly abnormal. This applied especially to the material as a whole but also to the group of patients who according to the case history had been most severely affected. It was found also that it was not always in the patients who had had especially severe symptoms that the values showed the greatest difference from the normal values. It was therefore often of interest to make a separate study of those patients in whom this difference was found to be greatest. This group, however, exhibited a status that in many respects could be regarded as remarkably good in consideration of the fact that this material was composed of persons who during often large parts of their lives had been completely or partly disabled by asthma symptoms. It therefore seemed reasonable to attribute the relatively small extent of the abnormal deviations in the hemodynamic findings to the fact that the dilatation of the terminal bronchioles indicated by the increased residual volume was not followed by destruction in the bronchial walls of such a degree as to cause irreversible damage. The reason that in these asthma patients in spite of the often greatly increased residual volume there did not appear to be any great functional consequences of the disease was assumed to be that the material did not include patients in whom the asthma was complicated by chronic bronchitis. It is considered probable that when bronchial asthma is com-

ed a significant decrease in the mean pressure in the pulmonary artery while the cardiac minute volume was essentially unchanged. The effect of breathing oxygen in patients with chronic bronchitis, some of whom had pulmonary emphysema has been studied by Cotes, Pisa and Thomas (1963). These authors found a tendency to a lower mean pressure and a reduced cardiac output during oxygen breathing than during the breathing of air.

In the present investigations a moderate mean increase in the cardiac minute volume was obtained both during hyperoxic breathing and during

acetylcholine infusion, in comparison with ordinary resting conditions. The pressure conditions in the pulmonary artery remained essentially unchanged, which was to be expected since the material consisted of normotensive patients.

During hypercapnoea a moderate mean increase in both the cardiac minute volume and the pressure in the pulmonary artery was recorded, in comparison with ordinary resting conditions. The results cannot be claimed with certainty however to support the view that pulmonary vasoconstriction was provoked.

### Summary

There appear to have been no systematic studies made previously on the effects of uncomplicated bronchial asthma on the cardiac and pulmonary functions. By uncomplicated bronchial asthma is meant that there is no evidence that chronic bronchitis — which condition has been defined — is simultaneously present or has previously been present. Neither does there appear to have been any study made previously in which a systematic attempt was made to assess the relationship between different factors characteristic of the case history of an asthma patient and the status exhibited by a group of such patients with regard to ventilation capacity, lung volumes, physical work capacity and hemodynamic function.

The results are given of an investigation (carried out mainly during the

period August 1961—March 1963) on the long term effects of bronchial asthma on the respiratory and cardiovascular functions in a series of patients who had lived in a given region during a certain period, and who during this period had been admitted to hospital in Uppsala for treatment of this disease. Of the 128 patients studied, 12 were diagnosed as having or having had — in addition to the bronchial asthma — a chronic disease which it was considered might have had a decisive influence on the cardio-respiratory functions. These patients were not included in the general analyses of the results of indices of the different functions studied. Because of the possible influence of the super-vening chronic disease this group is reported separately.

The degree of severity of the asthma

creasing age, and in this respect a higher age at onset might be of greater disadvantage than a lower for a given duration of the disease.

On studying the influence of different factors in the case history on the functional status at the time of the present investigation, certain findings appeared to indicate that the influence of the asthma on some essential functions was of a reversible nature. In order to study this question further a group of patients with a relatively high disease intensity during the years almost immediately preceding the time of the investigation was compared with a group in which the intensity of the disease during the same period was relatively low. Comparison was also made between a group of patients with a high over all degree of severity and a group in which this was low. The first comparison but not the second, showed a significant difference between the two groups as regards the function variable studied.

In the present investigations on pulmonary arterial blood pressure and pulmonary circulation it was seen that during resting conditions this series of patients exhibited a status that differed to only a relatively small extent from normal values. To a certain — but considerably lesser — extent this may also be said to hold for these function variables during physical exercise. In order to further provoke — in addition to the graded work test — any possible abnormal reactions that were not revealed during a resting state the anoxic anoxia test was carried out. In a further effort to illustrate the consequences of bronchial asthma for this

series of patients, pressure and flow studies were made during heart catheterization (to be published) on a randomly selected number of patients from the series in 58 patients during the inspiration of a mixture of 50 % oxygen in nitrogen, in 40 patients during the inspiration of 5 %  $\text{CO}_2$  in air and in 19 patients during continuous infusion of acetylcholine into the pulmonary artery at a dose rate of 11 mg/min. Such tests have to be evaluated with caution, as circulatory or respiratory steady state during the period of measurement cannot be guaranteed (for methods, see page 28) — which makes determinations of cardiac output by the Fick principle uncertain. The general circulatory reaction nevertheless seems of interest, and a preliminary summary of the results is therefore given here.

As no studies appear to have been made previously on the effect of hyperoxic breathing acetylcholine infusion or hypercapnoea in patients with uncomplicated bronchial asthma these examinations were considered warranted. The effect of hypercapnoea on the pulmonary circulation in patients with pulmonary emphysema has been studied by among others, Fishman, Fritts and Courmand (1960) who found a significant increase in both the cardiac minute volume and the mean pressure in the pulmonary artery during the inspiration of 3–5 %  $\text{CO}_2$  in air. The effect of an infusion of acetylcholine during a resting state in patients with pulmonary emphysema and pulmonary hypertension has been studied among others, by Behnke, Williams and White (1963) who show

ed a significant decrease in the mean pressure in the pulmonary artery while the cardiac minute volume was essentially unchanged. The effect of breathing oxygen in patients with chronic bronchitis, some of whom had pulmonary emphysema, has been studied by Cotes, Piza and Thomas (1963). These authors found a tendency to a lower mean pressure and a reduced cardiac output during oxygen breathing than during the breathing of air.

In the present investigations a moderate mean increase in the cardiac minute volume was obtained both during hyperoxic breathing and during

acetylcholine infusion, in comparison with ordinary resting conditions. The pressure conditions in the pulmonary artery remained essentially unchanged, which was to be expected since the material consisted of normotensive patients.

During hypercapnoea a moderate mean increase in both the cardiac minute volume and the pressure in the pulmonary artery was recorded, in comparison with ordinary resting conditions. The results cannot be claimed with certainty however to support the view that pulmonary vasoconstriction was provoked.

### *Summary*

There appear to have been no systematic studies made previously on the effects of uncomplicated bronchial asthma on the cardiac and pulmonary functions. By uncomplicated bronchial asthma is meant that there is no evidence that chronic bronchitis — which condition has been defined — is simultaneously present or has previously been present. Neither does there appear to have been any study made previously in which a systematic attempt was made to assess the relationship between different factors characteristic of the case history of an asthma patient and the status exhibited by a group of such patients with regard to ventilation capacity, lung volumes, physical work capacity and hemodynamic function.

The results are given of an investigation carried out mainly during the

period August 1961—March 1963) on the long term effects of bronchial asthma on the respiratory and cardiovascular functions in a series of patients who had lived in a given region during a certain period and who during this period had been admitted to hospital in Uppsala for treatment of this disease. Of the 128 patients studied, 12 were diagnosed as having or having had — in addition to the bronchial asthma — a chronic disease which it was considered might have had a decisive influence on the cardio-respiratory functions. These patients were not included in the general analyses of the results of indices of the different functions studied. Because of the possible influence of the super-vening chronic disease, this group is reported separately.

The degree of severity of the asthma



in the series of patients studied here varied greatly. The patients were therefore divided into groups according to the estimated degree of severity of the disease and the investigation findings were then compared between these groups. In addition comparisons were also made between the asthma material—or its groups—and control material.

The tests which measure ventilation capacity showed, together with the ratio of the residual volume to the total lung capacity the greatest mean percentage deviations from the predicted value. The smallest deviations were noted for the total lung capacity and the vital capacity. There appeared to be some relationship—as expected—between the estimated degree of severity of the disease and the size of both the ratio  $RV/TLC$  and the ratio  $FRC/TLC$ . In the asthma series on comparison with the control material the mean ratio  $RV/TLC$  in particular showed a highly significant difference.

The value for the material as a whole differed on an average highly significantly from the predicted value in all of the tests used as indices of the ventilation capacity. With respect to each of the tests significant mutual differences were shown between the asthma groups formed according to the estimated degree of severity of the disease; i.e. there was a significant difference between the mean value for those patients with fewer than 1000 total sickness points and that for the patients with a higher number. The value for the material as a whole differed on an average highly significantly from the predicted value (accord-

ing to the control material) for the ratio of both the residual volume and the functional residual capacity to the total lung capacity.

Regression analyses of the importance of different case history factors to the ventilation capacity showed that the intensity of the disease during the five-year period almost immediately preceding the time of the investigation was of relatively great influence compared with the over all severity since the onset of the asthma. This appears to support the view that the influence of the disease on the ventilation capacity is of a reversible nature when the disease becomes less active. The regression analysis showed, however, that the relation between the ratios of the residual volume and the functional residual capacity to the total lung capacity and the intensity of the disease during the almost immediately previous years was relatively small. On the other hand there was a closer relationship between the ratio of the residual volume in particular to the total lung capacity and the over all severity of the disease since the onset of the asthma. This suggests that the present study has offered some support for the view that in uncomplicated bronchial asthma hyperinflation does not appear to be characterized by reversibility to any great extent.

The mean value for the observed physical work capacity in the asthma patients differed significantly from the predicted value. It was considered that this might be due to a certain extent to the alteration, from a physical aspect, in the way of living; i.e. an increase in the circulatory functional

capacity was produced because of lack of exercise which appeared to have resulted from the disease in the majority of the patients. On classification of the patients into groups according to the estimated degree of severity of the asthma, it was found that among the sub-groups formed with regard to several case history factors there was a significant difference between the mean calculated values for the physical work capacity. The influence of the disease, measured by sickness points, on the physical work capacity appears to be exerted to a great part via the influence on the ventilation capacity.

A significant relationship was found for the patients in the asthma series between, on the one hand, both vital capacity and ventilation capacity as measured by the tests of  $FEV_{1.0}$ ,  $FEV_{1.5}$ ,  $MMV_{60}$  or  $MMV_1$  and, on the other hand, physical work capacity as measured by  $W_{120}$  or  $W_{max}$ . The relationship between physical work capacity and the ratio of residual volume to total lung capacity appeared to be negligible.

No definite signs of cor pulmonale — definition according to WHO Committee on Chronic Cor Pulmonale (1961) — were found in any of the patients in this series. All those on whom heart catheterization was performed had normal pressures in the lesser circulation at rest. The disease in these patients therefore differed, with regard to the pressure conditions in the pulmonary artery from the series of patients, reported from other countries with chronic obstructive pulmonary disease, and with some tendency to pulmonary hypertension. A probable explanation

is that this material is considered to represent the course in uncomplicated bronchial asthma while in the other series of cases various other factors such as chronic infection and air pollution were involved. It seems reasonable to conclude therefore that bronchial asthma does not as a rule lead to pulmonary hypertension if it is not complicated by other diseases leading to lung tissue destruction. The series of patients reported here showed on an average a moderate tendency to an increased ratio of the residual volume to the total lung capacity but since there was no pulmonary hypertension during a normal resting state it was improbable that this meant the presence of extensive destructive pulmonary emphysema. It is more likely that the increase was due only to a slightly complicated state of hyperinflation.

During physical exercise, however the pressure in the pulmonary artery rose to abnormal values in some patients. The pressure during exercise was found to have some relationship with the estimated degree of severity of the disease. This relationship was significant for the intensity of the disease during the five years almost immediately preceding the time of the investigation. It was not significant, on the other hand, when the over all degree of severity since the onset of the asthma was taken into account. This supports the view that bronchial asthma that has been intensive for a few years does have an effect on the pulmonary circulation even during a symptom free state. This influence was not shown during a resting state, but

only when physical exercise was begun. There appears to be some indication that the pressure increase is of a reversible nature, i. e. it decreases or disappears when the disease becomes less active.

The results of the investigation appeared to show that the orthostatic effect on the circulation in both the standing and the sitting positions as compared to the supine position was of a normal order of size in the asthma material.

The stroke volume at rest in the asthma patients was on an average somewhat lower compared with normal control persons. On changing over from a resting state to physical work the asthma patients showed on an average a relatively great increase in the stroke volume. The arterio-venous oxygen difference in relation to oxygen uptake was on an average higher in the asthma series than in the control material — which mainly consisted of younger male persons. A "hypokinetic circulation" was not judged to be present however as on an average the arterio-venous oxygen difference in relation to heart rate, as well as the increase of cardiac output in relation to increase of oxygen uptake during muscular work did not deviate from that of the control material. The asthma patients were therefore from this point of view regarded as "normokinetic".

The relationship between pairs of different function variables was studied. In the asthma material there appeared to be some relationship between several of these variables, and in some pairs this relationship was significant.

The function variable which on being paired with others showed the lowest mean correlation was the ratio  $RV/TLC$ . In consideration of the relatively large increase in this ratio shown on an average by these patients a relationship with other variables would not have been unexpected. In this material no relationship was found between  $RV/TLC$  ratio and the pressure in the pulmonary artery or the pulmonary vascular resistance, not even when the two latter were calculated during physical exercise. This probably indicates that in patients with a high  $RV/TLC$  ratio hyperinflation complicated with alveolar tissue destruction occurred only to a small or moderate extent. It is considered possible that the reason may be found here for the fact that the hemodynamic findings were abnormal only to such a small extent in these patients. This fact is valid for the material as a whole, but also to a certain extent for the group of patients in whom the severity of the asthma was assessed as pronounced. It was found, however, that it was not always the values in patients with a history of particularly severe asthma that differed most greatly from the normal values of the function variables. For this reason the patients who for certain function variables were found to show the greatest difference were studied separately. This group of patients also exhibited a status that in several respects could be regarded as remarkably good considering that this material was composed of patients who during often great parts of their lives had been completely or partly disabled by asthma symptoms.

## Mortality

Since in this investigation the clinical course was not subjected to an independent and complete analysis, the mortality study was included in the socio-medical analysis and discussion. The mortality was studied both for the total asthma series and also for those patients who, on their first admission to hospital, were resident in the Uppsala urban district.

*Mortality study on the total asthma series* i.e. all those patients who on admission to hospital fulfilled the original criteria for inclusion in the material.

For a detailed significance analysis of the mortality the whole of the asthma series, i.e. 170 patients, were included. The calculations were made according to the principles described in *Statistical Methods* (page 158). The expected number of deaths according to the risk figures for Swedish towns was 5.31 for men and 8.00 for women (see Table 48). The risk figures for Swedish towns were used. This implies an approximation because only a few more than 80% of the patients in the asthma series be-

longed to the town population. The actual number of deaths among the 18 cases of the asthma series—i.e. all those who on admission to hospital fulfilled the criteria for inclusion in the material—was 15 (8 men and 7 women). The difference from the expected number did lie in the predicted direction but was not significant.

It should be noted that the mortality for both the female and male population of the whole country is in close agreement with that for the towns alone. For the male population there is some degree of excess mortality for the towns, especially in the ages over 60 years. For the females on the other hand, there was somewhat lower mortality. If the mortality for the town population had been replaced by that for the population of the whole country the mortality observed in the asthma series would be practically the same.

16 of the 18 cases (3 men and 3 women) the deaths are directly related to the asthma attack. Of the remaining ten patients one man with bronchiectasis and history of chronic bronchitis died of renal and circulatory insufficiency after an attack of asthma and broncho-pneumonia which latter had been difficult to master clinically. Four patients died of cerebral haemorrhage, cardiac infarct, diabetic gangrene and cancer of the liver respectively. One patient died post-operatively after bilateral thyroidectomy; the cause of death was considered to be acute teleostasis, which affected both lungs simultaneously. One

only when physical exercise was begun. There appears to be some indication that the pressure increase is of a reversible nature, i. e. it decreases or disappears when the disease becomes less active.

The results of the investigation appeared to show that the orthostatic effect on the circulation in both the standing and the sitting positions as compared to the supine position was of a normal order of size in the asthma material.

The stroke volume at rest in the asthma patients was on an average somewhat lower compared with normal control persons. On changing over from a resting state to physical work the asthma patients showed on an average a relatively great increase in the stroke volume. The arterio-venous oxygen difference in relation to oxygen uptake was on an average higher in the asthma series than in the control material — which mainly consisted of younger male persons. A "hypokinetic" circulation was not judged to be present; however, as on an average the arterio-venous oxygen difference in relation to heart rate, as well as the increase of cardiac output in relation to increase of oxygen uptake during muscular work did not deviate from that of the control material. The asthma patients were therefore from this point of view regarded as "normokinetic".

The relationship between pairs of different function variables was studied. In the asthma material there appeared to be some relationship between several of these variables, and in some pairs this relationship was significant.

The function variable which on being paired with others showed the lowest mean correlation was the ratio  $R_V/TLC$ . In consideration of the relatively large increase in this ratio shown on an average by these patients a relationship with other variables would not have been unexpected. In this material no relationship was found between  $R_V/TLC$  ratio and the pressure in the pulmonary artery or the pulmonary vascular resistance, not even when the two latter were calculated during physical exercise. This probably indicates that in patients with a high  $R_V/TLC$  ratio hyperinflation complicated with alveolar tissue destruction occurred only to a small or moderate extent. It is considered possible that the reason may be found here for the fact that the hemodynamic findings were abnormal only to such a small extent in these patients. This fact is valid for the material as a whole but also to a certain extent for the group of patients in whom the severity of the asthma was assessed as pronounced. It was found, however, that it was not always the values in patients with a history of particularly severe asthma that differed most greatly from the normal values of the function variables. For this reason the patients who for certain function variables were found to show the greatest difference were studied separately. This group of patients also exhibited a status that in several respects could be regarded as remarkably good considering that this material was composed of patients who during often great parts of their lives had been completely or partly disabled by asthma symptoms.

## VIII SOCIO-MEDICAL ASPECTS

### Mortality

Since in this investigation the clinical course was not subjected to an independent and complete analysis, the mortality study was included in the socio-medical analysis and discussion. The mortality was studied both for the total asthma series and also for those patients who, on their first admission to hospital, were resident in the Uppsala urban district.

*Mortality study on the total asthma series i.e. all those patients who on admission to hospital fulfilled the original criteria for inclusion in the material*

For a detailed significance analysis of the mortality the whole of the asthma series, i.e. 176 patients, were included. The calculations were made according to the principles described in *Statistical Methods* (page 168). The expected number of deaths according to the risk figures for Swedish towns was 5.31 for men and 5.00 for women (see Table 48). The risk figures for Swedish towns were used. This implies an approximation because only a few more than 80% of the patients in the asthma series be-

longed to the town population. The actual number of deaths among the 176 cases of the asthma series—i.e. all those who on admission to hospital fulfilled the criteria for inclusion in the material—was 15 (8 men and 7 women). The difference from the expected number did lie in the predicted direction but was not significant.

It should be noted that the mortality for both the female and male population of the whole country was in less agreement with that for the towns alone. For the male population there was some degree of excess mortality for the towns, especially in the ages over 40 years. For the females, on the other hand, there was somewhat lower mortality. If the mortality for the town population had been replaced by that for the population of the whole country the mortality observed in the asthma series would be practically the same.

7 of the 15 cases (2 men and 5 women) the deaths were directly related to a thoracic attack. Of the remaining ten patients one man with bronchiectasis and a history of chronic bronchitis died of renal and circulatory insufficiency after an attack of asthma and broncho-pneumonia which latter had been difficult to master clinically. Four patients died of cerebral haemorrhage, cardiac infarct, diabetic gangrene and cancer of the liver respectively. One patient died post-operatively after bilateral thyroidectomy; the cause of death was considered to be acute tubercula, which affected both lungs simultaneously. One

elderly man died at home—the cause of death was unknown. The cause of another patient's death was iatrogenic—not connected, however with the treatment of asthma. In two or possibly three cases the direct cause of death was considered to be broncho-pneumonia. In at least one of these cases it was believed that large doses of corticosteroids had contributed greatly not only to the occurrence of the pneumonia, but also to the fact that the latter was not mastered by the use of antibiotics and chemo therapy.

*Mortality study on those patients in the asthma series who on their first admission to hospital were resident in the Uppsala urban district*

As a complement to the study of change of residence (see below) an investigation was made of the mortality of the urban district patients.

Table 47 shows the survival probability figures for the 10 year period 1951—1960 (see mortality and life-expectancy tables in the official statistics for Sweden) for the Swedish town population in the respective age and sex groups. Also shown in the table is the percentage of surviving male Uppsala town residents in the asthma series; this figure was 88.5 (40/52) which may be compared with the corresponding figure for the Swedish towns which for a male population with the same age grouping as that of the asthma series was 92.4. The corresponding survival percentages for women were 92.3 (84/91) (asthma material) and 95.6 (the female town population). Thus the mortality appeared to be higher in the asthma series but the difference was not significant.

To summarize it may be said that the observed mortality in the asthma series was somewhat higher than the expected. This difference, however was not significant.

### Invalid pension

The studies made of the social situation of this group of asthma patients were based on information that was valid for the 1st November 1960.

Of the 116 patients on whom the main proportion of the general analyses were based, 10 men and 11 women were receiving an invalid pension at this date. The main reason in these patients was bronchial asthma. It was found that the 10 men received their pension at a mean age of 52 years (range 25—66 years). The corresponding figure for the 11 women was 51 years (range 27—66 years).

Apart from these 21 patients who received an invalid pension, two women were also given an early pension by their employers on account of bronchial asthma. In one case this was received at the age of 48 years, and in the other at 54 years. Since, however these two patients were not assessed in accordance with the norms of the National Social Insurance Board, they were not included as invalid pensioners in the analyses.

Of those who had other chronic diseases besides the bronchial asthma which were assessed as having adverse influence on their life situation, their general condition and functions, six patients were receiving an invalid pension. Of these, three (two men, Nos. 66 and 118, and one woman, No. 45) were receiving a pension because of bronchial asthma. The other three (one man, No. 112 and two women, Nos. 77 and 101) were receiving a pension on account of cerebral thrombosis, pulmonary tuberculosis and cardiac

arteriosclerosis, respectively. For the case histories of these patients reference may be made to page 100.

### Change of residence

The frequency of change of residence was studied for those patients in the asthma series who on their first admission to hospital were resident in the urban district of Uppsala. A comparison was made with the total population of Uppsala.

### The Uppsala urban population

The Uppsala urban population, and its distribution into sex and age were known for the end of both 1950 and 1960. By selection from the 1961 population schedule (which was also for the end of 1960) the number of those who lived in Uppsala at the end of 1950 and were still resident there at the end of 1960 was estimated. Every 36th person was studied—one person on every page of the

population schedule. The result of this selection study is shown in Table 45. By multiplying the number of persons selected by 36, an estimate of the total number of those who lived in Uppsala in 1950 and were still resident there in 1960 was obtained.

A calculation was then made of the percentage of the Uppsala urban population at the end of 1950 constituted by those still resident in Uppsala at the end of this ten-year period (see Table 46, columns I and V). The percentage of those still resident in the town can be estimated in two ways. The estimate shown in column IV Table 46, was obtained by multiplying the number still resident, according to the selection study by 36 (see Table 45, column I) and Table 46, column II) and dividing it by the number of persons resident in Uppsala in 1950 (see Table 46 column I). A better assessment is shown in column V Table 46. This takes into account the fact that the age and sex distribution in the selected persons was not identical with that shown by the 1960 population census (cf columns III and V in Table 45). Firstly an estimate was made of the number of persons who were resident in Uppsala both in 1950 and 1960 (see Table 45, column III) then

Sex	Age group yrs.	Birth yr. group	Selection		36 selection		Resident in Uppsala at end of 1960 or acc. to population census
			Resident in Uppsala at end of 1960	Of whom also resident in Uppsala at end of 1950	Resident in Uppsala at end of 1960	Of whom also resident in Uppsala at end of 1950	
			I	II	III	IV	V
Male	20—24	1936—1939	74	32	2664	1142	2725
	25—29	1921—1925	73	38	2628	1388	2601
	30—34	1916—1920	87	70	3132	2536	2513
	35—39	1911—1915	64	44	2304	1664	2197
	40—44	1906—1910	69	48	2484	1728	2226
	45—49	1901—1905	42	31	1512	1116	1836
	50—54	1896—1900	44	26	1584	1296	1620
	55—59	1891—1895	20	15	720	540	855
Female	20—24	1936—1939	87	31	3132	1116	2709
	25—29	1921—1925	71	43	2556	1548	2620
	30—34	1916—1920	85	87	3060	3032	2762
	35—39	1911—1915	86	63	3108	2268	2553
	40—44	1906—1910	78	53	2808	1908	2387
	45—49	1901—1905	62	47	2232	1692	2122
	50—54	1906—1900	84	37	3024	1332	2161
	55—59	1891—1895	28	18	1008	684	1269

Table 45. Result of selection study comprising every 36th person in the Uppsala population selected for 1961 (referring to end of 1960).



$$\text{Column III in Table 46} = \frac{(\text{Column II in Table 45})}{(\text{Column I in Table 45})} \quad (\text{Column V in Table 45})$$

and then we obtain

$$\text{Column V in Table 46} = \frac{(\text{Column III in Table 46})}{(\text{Column I in Table 46})}$$

For the group of persons born in 1800—1900 for example, it was found that about 80 % of the men belonged to those who were still resident in Uppsala in 1960 while the corresponding figure for the women was 70 %. For the youngest age group of those born in 1926—1930 these figures were 51 % and 44 % respectively. As Table 46 shows, a great number had moved to other districts. It is evident that on the whole the percentage number of those still resident increased with increase in age.

### The asthma series

The number of patients who lived in Uppsala at the time of their admission to hospital was 143 and for this group distributed according to age and sex, Table 47 gives the following information

- 1) the number who on admission to hospital fulfilled the criteria for inclusion in the material (column D)
- 2) the number who had died before the 1st November 1960 (column A)
- 3) the number out of those who were still alive on the 1st November 1960 who had moved to another district before this date (column B)
- 4) the number who were still resident in Uppsala on the 1st November 1960 (column C)
- 5) the mean year in which the patients were first admitted to hospital (column E)

Regarding the material as a whole, it was found that the number of men still resident in Uppsala on the 1st November 1960 was 30/52 and the number of women 73/91. The mean "first admission" year for the whole material was 1950.8 thus the mean observation period in this material was almost exactly ten years. By "first admission" is meant the first time a patient was ad-

Year of birth	MALE					FEMALE				
	Resident in Uppsala 1950	Resident in Uppsala both 1950 and 1960		% still resident		Resident in Uppsala 1950	Resident in Uppsala both 1950 and 1960		% still resident	
	I	II	III	IV	V	I	II	III	IV	V
1926—1930	2282	1152	1178	50.5	51.0	283*	1116	1233	39.4	41.2
1921—1925	2700	1368	1351	49.0	48.5	3126	1518	1708	49.5	54.0
1916—1920	2180	2520	2022	97.3	78.1	2803	2032	1832	1	61
1911—1915	2201	1584	1510	70.0	66.8	2309	2268	1827	99.4	2.8
1906—1910	2347	1728	1556	6.9	69.2	2150	1908	157	7.6	1.3
1901—1905	1832	1110	1353	60.0	74.0	2261	1602	1836	4	81.1
1896—1900	1614	1298	1325	78.8	80.6	1996	1332	1413	66.7	70.8
1893—1895	1010	840	672	53.5	66.5	1220	681	820	56.1	67.2

T ble 46. E: estimated number and percentage of the 1950 population still resident in Uppsala in 1960

The calculation of the percentage still resident was made in two ways (cf Table 4). Column V in Table 4 gives the best estimate of the percentage still resident.  
approximate number

mitted to hospital for asthma during the years 1915—1958. For many age groups, however, this observation period was shorter or longer than ten years. The percentage number of those still

resident was therefore corrected to correspond with an observation period of ten years (see column F). The correction formula used is given at the head of column F.

Sex Year of birth	Asthma patients resident in Uppsala at first admission to hospital						Uppsala population still resident in Uppsala after 1928 or in Col. V in Table 2	Expected percent- age survivors during the 10-yr period 1928-37 for period, see Table 2	Difference F-G
	Died before 1915	ADys L11 88 who moved to another district before 1915	Still resident in Uppsala 1915	Total A+B+C	First admission year (mean)	Percentage still resident corrected with regard to ad- mission year see to the formula $\frac{10(D-C)}{D(1900-62-2)}$			
	A	B	C	D	E	F			
<b>MALE</b>									
1928-1930	0	1	5	6	19.07	83.6	31.6	83.9	52.0
1931-1933	1	3	2	6	19.18	43.9	48.5	98.6	-2.6
1934-1936	0	2	2	4	19.28	82.5	74.1	94.1	-25.8
1937-1939	0	0	5	5	19.34	100.0	66.8	87.2	33.2
1940-1942	0	0	4	4	19.38	100.0	64.3	84.5	30.8
1943-1945	1	0	7	8	19.43	84.9	74.0	92.7	12.9
1946-1948	3	1	5	8	19.50	57.5	60.0	84.8	-23.1
1949-1951	2	0	0	11	19.56	77.0	66.3	82.0	11.4
Total Male	6	7	20	32	19.13				
Standard weighted percentage figures (Col. D stand pop.)						73.0	67.2	92.4	7.8
<b>FEMALE</b>									
1928-1930	0	3	8	9	19.11	65.7	44.2	97.4	21.5
1931-1933	0	3	5	10	19.19	48.1	34.0	91.1	3.5
1934-1936	2	0	4	6	19.26	62.2	61.7	98.7	2.5
1937-1939	1	1	18	19	19.33	86.2	72.8	97.0	13.4
1940-1942	0	0	6	6	19.40	100.0	1	98.0	28.5
1943-1945	3	1	14	18	19.50	78.0	81.1	93.3	-2.1
1946-1948	0	0	17	17	19.57	100.0	70.8	82.1	29.3
1949-1951	1	1	9	11	19.63	83.8	67.2	80.2	16.6
Total Female	7	11	73	91	19.53				
Standard weighted percentage figures (Col. D stand pop.)						81.1	67.9	93.6	13.2

The 47 asthma patients who died, moved from and still lived in Uppsala respectively classified according to sex and age. The percentages still resident in Uppsala respectively classified Uppsala population. Expected mortality according to the mortality and life expectancy tables for the town population in Sweden for the 10-yr period 1937—50.

Two women were born after 1930. They are combined in this and the following tables showing age distribution with the patient born in 1930.

Column III in Table 46 =  $\frac{(\text{Column II in Table 43})}{(\text{Column I in Table 43})}$  (Column V in Table 43)

and then, we obtain

Column V in Table 46 =  $\frac{(\text{Column III in Table 46})}{(\text{Column I in Table 46})}$

For the group of persons born in 1896—1900 for example, it was found that about 80 % of the men belonged to those who were still resident in Uppsala in 1960 while the corresponding figure for the women was 70 %. For the youngest age group of those born in 1926—1930 these figures were 51 % and 44 % respectively. As Table 46 shows a great number had moved to other districts. It is evident that on the whole the percentage number of those still resident increased with increase in age.

### The asthma series

The number of patients who lived in Uppsala at the time of their admission to hospital was 143 and for this group distributed according to age and sex, Table 47 gives the following information

- 1) the number who on admission to hospital fulfilled the criteria for inclusion in the material (column D)
- 2) the number who had died before the 1st November 1960 (column A)
- 3) the number out of those who were still alive on the 1st November 1960 who had moved to another district before this date (column B)
- 4) the number who were still resident in Uppsala on the 1st November 1960 (column C)
- 5) the mean year in which the patients were first admitted to hospital (column E)

Regarding the material as a whole it was found that the number of men still resident in Uppsala on the 1st November 1960 was 30/52, and the number of women 73/91. The mean first admission year for the whole material was 1950.8 thus the mean observation period in this material was almost exactly ten years. By first admission is meant the first time a patient was ad-

Year of birth	MALE					FEMALE				
	Resident in Uppsala 1950	Resident in Uppsala both 1950 and 1960		% still resident		Resident in Uppsala 1950	Resident in Uppsala both 1950 and 1960		% still resident	
	I	II	III	II/I IV	III/I V	I	II	III	II/I IV	III/I V
1926—1930	2282	1182	1178	50.5	51.6	2832	1110	1253	39.4	44.2
1921—1925	2790	1368	1351	49.0	48.8	3126	1648	1708	49.5	54.6
1916—1920	2580	2520	2022	97.3	78.1	2863	2032	1852	71	61
1911—1915	2201	1581	1510	70.0	68.8	2509	2268	1827	90.4	72.8
1906—1910	2217	1728	1556	78.0	69.2	2159	1908	1757	88.4	81.3
1901—1905	1832	1116	1355	60.9	74.0	2281	1692	1856	74.2	81.4
1896—1900	1611	1296	1325	78.8	80.6	1906	1332	1413	69.9	74.2
1891—1895	101	510	672	53.5	66.5	1220	681	820	56.1	67.2

Table 40. E. Estimated number and percentage of the 1950 population still resident in Uppsala 1960

The calculation of the percentage still resident was made in two ways (cf Table 46). Column V shows the best estimate of the percentage still resident, approximate number

for the matriculation examination were 0.9 and 2, respectively. Even if the difference was not significant there was a tendency towards a greater number of examinations passed in the asthma series. On assessing these figures, however, it must be taken into account that the control figures applied to the whole of Sweden, and the total population in the collection area from which the patients in the asthma series were taken probably did not show agreement with the total Swedish population in this respect. Uppsala — a long standing school and university town — may be assumed to show some overrepresentation with regard to both the lower certificate and the matriculation examination.

In order to find out how the patients in the asthma series compared with the total urban population of Uppsala, the material was selected in the same way as above but with the exclusion of the three patients from the rural districts. A study was made of the incidence of persons with an education comprising matriculation examination plus a university degree or their equivalents. The expected number of such persons was calculated from the 1960 population census for the urban district of Uppsala. Consideration was taken of age and sex. The expected number with this education was found to be 1.7 and the observed number 2.

*T. summary.* It may thus be said that from the point of view of education the selected patients in the asthma series did not differ significantly from the total population. Some tendency towards a greater number of examinations passed was observed in

the asthma patients (see Table 48). The question of whether or not this tendency can be assumed to have any relationship to the disease will be discussed later.

#### *Family data*

*Marriage frequency.* The marriage frequency was estimated both in the whole main asthma series, i.e. those patients still alive and still resident in Uppsala on the 1st November 1960 (116 patients) and — in order to study the influence of the disease on this frequency — in a selection of 20 patients from this series, in whom the onset of asthma occurred at or before the age of 21 years and who up to the age of 30 years had had a maximum of two symptom free calendar years. In both cases the expected number of persons who had at any time been married was calculated with the aid of information from the 1960 population census, consideration being taken of sex, age and district of residence, i.e. whether the persons in question had lived in the urban or the rural districts of Uppsala. The expected number of those who had at any time been married, in the whole main series, was 98.0 while the observed number was 101. The corresponding figures for the selected series were 24.6 and 24 respectively. The patients in the asthma material did not thus appear to differ from the total population in this respect (see Table 48).

*Divorce.* The number of divorces was studied in the main asthma series, i.e. those patients still alive and still resident in the collection area on the 1st November 1960 and who at any

The figures for those still resident in Uppsala on the 1st November 1960 appeared to be higher in the asthma series than in the Uppsala population. The age distribution of the asthma series was not the same as that of the Uppsala population. The older age groups were over represented in the asthma series and this age group in particular showed according to Table 46 the highest percentage figures for persons still resident in Uppsala at the given date. Standard weighting was therefore performed, column D constituting the standard population.

Besides the data concerning the asthma series Table 47 shows in column G the percentage number of the 1950 Uppsala population still resident in this town on the 1st November 1960 (cf Table 46 column V).

It was found that the standard weighted percentage figures for those still resident in Uppsala was higher for the asthma series the difference for the men being 7.8 % for the women 13.2 % and for the whole series, regardless of sex, 11.2 %. This latter difference is significant (\*\*). The higher percentage figure for the asthma series is especially surprising since the series exhibited a somewhat higher mortality than the total population of the Swedish towns. This emphasized the fact that the asthma patients showed a considerably lower tendency to move to other districts than the rest of the Uppsala population.

### General education and family data

Adolescence and the early adult years constitute the period when the basis for both education and family for-

mation is laid. Since in many of the patients in this series the age at the onset of asthma was relatively high, not all of the patients in the material were suitable for studies on the effect of the disease on these factors. It seemed desirable, however to illustrate as far as possible the significance of the disease from the point of view of social adaptation. Some questions of general education and family formation were therefore studied in a selected number of the more suitable patients from the series.

### General education

In order to obtain some idea of the influence of this disease on the general education of the patients, a selection was made of those patients in the series in whom the asthma was first manifested at or before the age of 16 years and who between the ages of 16 and 24 years had had a maximum of two symptom free calendar years. These criteria were fulfilled by 72 patients, 11 of each sex. 10 of the 22 lived in the urban district of Uppsala, and the other three in the rural districts. The expected number of persons passing the lower certificate and matriculation examinations was calculated from the Historical Statistics of Sweden. Consideration was taken of sex, age and the annual number of examinations passed in the whole country during the period when these patients were of the ages 15—19 years and the number of persons of this age group in Sweden at the time in question. The expected number of lower certificate examinations passed was 1.3 and the observed number 4. The corresponding figures

for the matriculation examination were 0.9 and 2 respectively. Even if the difference was not significant, there was a tendency towards a greater number of examinations passed in the asthma series. On assessing these figures, however it must be taken into account that the control figures applied to the whole of Sweden, and the total population in the collection area from which the patients in the asthma series were taken probably did not show agreement with the total Swedish population in this respect. Uppsala — a long standing school and university town — may be assumed to show some overrepresentation with regard to both the lower certificate and the matriculation examination.

In order to find out how the patients in the asthma series compared with the total urban population of Uppsala, the material was selected in the same way as above but with the exclusion of the three patients from the rural districts. A study was made of the incidence of persons with an education comprising matriculation examination plus a university degree or their equivalents. The expected number of such persons was calculated from the 1960 population census for the urban district of Uppsala. Consideration was taken of age and sex. The expected number with this education was found to be 1.7 and the observed number 2.

To summarize it may thus be said that from the point of view of education the selected patients in the asthma series did not differ significantly from the total population. Some tendency towards a greater number of examinations passed was observed in

the asthma patients (see Table 48). The question of whether or not this tendency can be assumed to have any relationship to the disease will be discussed later.

#### *Family data*

*Marriage frequency* The marriage frequency was estimated both in the whole main asthma series, i.e. those patients still alive and still resident in Uppsala on the 1st November 1960 (116 patients) and — in order to study the influence of the disease on this frequency — in a selection of 29 patients from this series, in whom the onset of asthma occurred at or before the age of 21 years and who up to the age of 30 years had had a maximum of two symptom free calendar years. In both cases the expected number of persons who had at any time been married was calculated with the aid of information from the 1960 population census, consideration being taken of sex, age and district of residence, i.e. whether the persons in question had lived in the urban or the rural districts of Uppsala. The expected number of those who had at any time been married, in the whole main series, was 98.0 while the observed number was 101. The corresponding figures for the selected series were 21.6 and 24, respectively. The patients in the asthma material did not thus appear to differ from the total population in this respect (see Table 48).

*Divorce.* The number of divorces was studied in the main asthma series, i.e. those patients still alive and still resident in the collection area on the 1st November 1960 and who at any

The figures for those still resident in Uppsala on the 1st November 1960 appeared to be higher in the asthma series than in the Uppsala population. The age distribution of the asthma series was not the same as that of the Uppsala population. The older age groups were over represented in the asthma series and this age group in particular showed according to Table 46 the highest percentage figures for persons still resident in Uppsala at the given date. Standard weighting was therefore performed, column D constituting the standard population.

Besides the data concerning the asthma series Table 47 shows, in column G the percentage number of the 1950 Uppsala population still resident in this town on the 1st November 1960 (cf Table 46 column V).

It was found that the standard weighted percentage figures for those still resident in Uppsala was higher for the asthma series the difference for the men being 7.8 % for the women 18.2 % and for the whole series, regardless of sex, 11.2 %. This latter difference is significant (\*\*). The higher percentage figure for the asthma series is especially surprising since the series exhibited a somewhat higher mortality than the total population of the Swedish towns. This emphasized the fact that the asthma patients showed a considerably lower tendency to move to other districts than the rest of the Uppsala population.

### General education and family data

Adolescence and the early adult years constitute the period when the basis for both education and family for-

mation is laid. Since in many of the patients in this series the age at the onset of asthma was relatively high, not all of the patients in the material were suitable for studies on the effect of the disease on these factors. It seemed desirable, however to illustrate as far as possible the significance of the disease from the point of view of social adaptation. Some questions of general education and family formation were therefore studied in a selected number of the more suitable patients from the series.

### General education

In order to obtain some idea of the influence of this disease on the general education of the patients, a selection was made of those patients in the series in whom the asthma was first manifested at or before the age of 16 years and who between the ages of 16 and 24 years had had a maximum of two symptom free calendar years. These criteria were fulfilled by 22 patients, 11 of each sex. 10 of the 22 lived in the urban district of Uppsala and the other three in the rural districts. The expected number of persons passing the lower certificate and matriculation examinations was calculated from the Historical Statistics of Sweden. Consideration was taken of sex, age and the annual number of examinations passed in the whole country during the period when these patients were of the ages 15—19 years, and the number of persons of this age group in Sweden at the time in question. The expected number of lower certificate examinations passed was 1.3 and the observed number 4. The corresponding figures

Factor studied	Category of patients studied in the arthritis material	N	Population from which the risk figures were taken for calculating the expected values	In calculating the expected values consideration was taken of	Observed No. in the arthritis material	Expected figure
Dead	total series	18	the population of Sweden	per sex	15	10.31
	all the 1 total series	71	— —	—	8	8.31
	all women in total series	103	— —	—	7	8.00
Was resident in Uppsala	all who on first admission to hospital were resident in Uppsala	143	Uppsala urban population 1930 and 1960	per 5 or 10-yr groups, sex	112*	96.1
Lower cert. Males examination	all those on first admission at or before 18 yrs; examination of 3 symptom-free yrs during the age 18-24	25	population of Sweden	No. of lower cert. Males examinations per sex during the period when the patients in question were 18-24 yrs. and the No. of individuals 5 years in this age group at the time in question, sex	4	1.3
Maternity home residence	— —	22	—	—	2	0.9
Education reporting matriculation + university degree	resident Uppsala; set of subjects 1 or before age of 18 yrs; one symptom-free yrs during the ages 18-24	19	Uppsala urban population 1960	per 5 or 10-yr groups, sex	2	1.7
Married + previously married	total by sex included in the main material	116	Uppsala urban and rural population 1960	per sex groups	101	88
	those of this total born thus before 18 before the age of 21 and who during the age of 20-24 had one symptom-free yrs	29	— —	— —	24	24.6
On first + previously married	total 1 case married in the main material	101	Uppsala urban and rural population 1960	per sex group	4	10.8
No. of children under 18 yrs living in house	married living together whose children born before 21 yrs. and who during the age 20-24 yrs. had one symptom-free yrs over born 1923 or later and the born 1921 or later those of the above who were born those of the above who were women	11	Uppsala urban and rural population 1960	per 5 or 10-yr groups women assigned to groups according to their standard age	22	17.2
	— —	8	— —	— —	10	8.7
	— —	8	— —	— —	13	8.5

Table 18. Results of calculations of mortality residence in Uppsala, school education, number married and divorced and the number of children under the age of 18 yrs. in patients in the arthritis series. The Table shows the respective comparative populations and also the factors taken into account in calculating the expected figures. The degree of significance is noted.



time had been married (101 patients). The expected number of those who in 1960 lived as divorced persons, was calculated with the aid of information from the 1960 population census, consideration being taken of sex, age and district of residence. The expected number of divorced persons was 5.5 and the observed number 2. The difference is significant (\*).

Apart from the two patients in the asthma series who in 1960 lived as divorced persons, a further two in this series had also at one time been divorced. In order to find out how great a proportion of the married inhabitants of Uppsala had previously been divorced, 1 558 persons of the age groups in question were selected at random from the records of the registrar's office. For each of these persons a note was made of whether or not they had previously been divorced. The probands were divided into sex and age groups. On the basis of this information the expected number of previously divorced — but later re-married — persons was calculated for the asthma series. This figure was 5.0. The difference between the expected and observed figures was not significant. The difference might have increased somewhat however if the patients living in the rural district had also been included since none of these had been divorced.

Thus the number of patients in the asthma series who were living as divorced or had previously been divorced but had later re-married was 4 compared with an expected figure of 0.5. This difference is significant (\*) (see Table 48).

*Number of children under the age of 16 years.* To obtain some idea of the fertility in the asthma material a study was made of the number of children under the age of 16 years in 1960 of married parents from the asthma series living with their husbands or wives respectively and in whom the asthma was first manifested at or before the age of 21 years and also who had had a maximum of two symptom free calendar years between the ages of 21 and 30 years. Women born in 1923 or earlier and men born in 1920 or earlier were not included; the information on the number of children for these patients could not be assessed since it was more probable that they could also have had children at or above the age of 16 years; the official statistics only provide information for children under 16 years. The expected number of children was calculated from the 1960 population census, consideration being taken of age and district of residence. In this calculation of the expected number of children the women were counted as being of the same age as their respective husbands. The expected number of children for these patients regardless of sex was 17.3 and the observed number 23. The corresponding figures for the men were 8.7 and 10 respectively and for the women 8.5 and 13 respectively (see Table 48). The difference was not significant but supported the view that the patients in the asthma material did not have fewer children than the total population.

*To summarize:* these patients did not appear to differ from the total population with regard to marriage frequency.

Factor studied	Category of patient studied in the study material	No.	Population from which the risk figures were taken (calculating the expected rates)	Calculating the expected rates (consideration taken of)	Observed No. - the actual	Expected figure
Died	total series	170	rural population of Sweden	age sex	15	10.31
	all men in total series	71	— —	— —	8	5.31
	all women in total series	103	— —	—	7	8.00
Still resident in Uppsala	all who on first admission to hospital were resident in Uppsala	143	Uppsala urban population 1948 and 1960	age in 3-4 groups, sex	112*	96.1
Lower cert. clinical examination	all those on first or before 16 yrs maximum of 2 symptom free yrs. during the age 16-21	—	population of Sweden	No. of lower cert. clinical examination period during the period when the patient in question was 15-19 yrs. and the % of end-stage in Sweden in this age group at the time in question, sex	4	1.3
Matriculation examination	— —	22	— —	—	2	0.8
Education requiring matriculation + an entry degree	resident Uppsala on first or before age of 16 yrs; maximum of 2 symptom-free yrs. during the ages 16-21	19	Uppsala urban population 1960	age in 3 or 10 groups, sex	2	1.7
Married + previously married	total who are included in the study material	110	Uppsala urban and rural population 1960	age in 3-4 groups	101	96
	those of this total whose illness began before the age of 21 yrs and who during the age 20-29 had maximum of 2 symptom-free yrs.	29	—	—	21	21.6
Divorced + previously divorced	total in the study material	101	Uppsala urban and rural population 1960	age in 3-4 groups	4	10.5
No. of children under 16 yrs living in home	married living together whose illness began before 21 yrs. and who during the age 20-29 yrs. had maximum of 2 symptom-free yrs. (men born 1921 later and women born 1921 or later)	11	Uppsala urban and rural population 1960	age 3 or 10-4 groups	23	17.3
	those of the above who are men	3	— —	— —	10	8.7
	those of the above who are women	8	— —	— —	13	8.5

Table 4. Results of calculations of mortality residence in Uppsala, school education, number of children under the age of 16 yrs. in patient in the study. The table shows the respective comparative populations and also the factors calculating the expected figures. The degree of significance is assessed.

and the number of children. There was a tendency to fewer divorces in the asthma series than in the total population. The difference between the observed and expected figures was significant.

### Wage-earning

On studying the consequences of asthma an attempt was made to obtain as much information as possible in order to elucidate the effect of the disease on the social situation of the patients. It was considered of interest to classify the patients according to their branch of industry and occupational position. An attempt was also made to find out how many had changed their occupations or had discontinued to earn an income by reason of asthma and also the incidence of income-earners among the married women living with their husbands.

### Distribution according to branch of industry

Table 49 shows the distribution into

different branches of industry of the economically active men and women in this series of cases, compared with the general population of the same district according to the population census 1960. The figures given apply to the 1st November 1960.

Economically active persons are — according to the population census 1960 — defined as those who during the so-called population census week, carried out income-earning work comprising at least half the normal working hours. Also regarded as economically active were those persons who had income-yielding work but because of temporary discontinuation — e.g. vacation, illness, military repatriation exercises, temporary unemployment etc. — were not working during the population census week. The discontinuation was regarded as temporary if the duration of the interval was not more than 3 months. The same criteria applied essentially to the patients in the asthma series.

The total number of inhabitants in the collection area was about 65,000. The number of persons who were born during the period 1893—1932 was approximately 4,000. Of these 81% lived in the urban and 18% in the rural district. The corresponding figures for the asthma series were 81% and 18% respectively.

Branch of industry	Male			Female		
	Asthma series 1960		General population in the district of the asthma series 1960	Asthma series 1960		General population in the district of the asthma series 1960
	No.	%		No.	%	
Agriculture, forestry work etc.	6	19.4	12.3			2
Manufacturing and handicraft trade	8	25.8	31.3	4	17.4	18.3
Building trades	3	9.7	15.5			1.1
Commerce	3	8.6	11.7	3	13.0	10.3
Transport and communication	2	6.5	8.9	1	4.3	2.1
Government administration	10	32.3	22.6	13	63.4	39.1

Fig. 49 Economically active males and females in the asthma series classified according to branch of industry compared with the corresponding figures for the general population in the same district 1960.

In calculating the percentage figures of the control material consideration was taken of sex and district of residence but not of age, since there is no noteworthy relationship between industrial branch and age in the district concerned here. As the table shows, the distribution of the women between the different branches of industry was on the whole the same in the asthma series as in the total population. In the male asthma patients there was an overrepresentation (non-significant) in the group of general administration, some degree of overrepresentation (non-significant) in the category of agriculture and forestry work, and some degree of underrepresentation (non-significant) in the groups of manufacturing handicraft and building trades, and commerce.

#### *Distribution according to occupational status*

For the 54 economically active patients the distribution between the two occupational positions employer and employed was studied. Of these 51 (31 men and 23 women) 43 were resident in the urban district of Uppsala and 12 in the rural districts. For all 54 the expected number of employers was calculated from information from the Uppsala 1960 population census. Consideration was taken of sex and age. It was found that in the asthma series there were 11 employers compared with the expected 4.8. This difference between the observed and expected figure was significant ( ). There was no noteworthy sex difference. When only those patients in the asthma series who

lived in the urban district of Uppsala were studied, the observed number of employers was 8 while the expected number was 3.6. In this case the difference between the observed and expected figure did not reach the significance level.

#### *Changes of occupation*

Table 50 shows the changes of occupation among the patients in the asthma series. Only first changes were considered in this analysis. 36 % of the men stated that they had had to change their occupation on account of asthma, while the corresponding figure for the women was 11 %. This figure applied to the 36 women who in 1960 or earlier had income yielding work comprising at least half the normal hours (25 women had such work in 1960 and 11 women had previously had such work).

The mean age at the first change of occupation was 38 years for the men and 44 years for the women. On comparing the number of economically active persons in the different branches of industry before and after the change of occupation, it was found that 6 of the 7 men who had previously earned their living by agriculture and forestry work had left this branch of industry. The greatest inflow to any branch was found in the group of general administration. Even if tendencies such as these might be expected in view of the general interchanges between the different branches of industry during the last few decades, those observed in the asthma series seemed to be especially marked. There was an obvious ten-

and the number of children. There was a tendency to fewer divorces in the asthma series than in the total population. The difference between the observed and expected figures was significant.

### Wage-earning

On studying the consequences of asthma an attempt was made to obtain as much information as possible in order to elucidate the effect of the disease on the social situation of the patients. It was considered of interest to classify the patients according to their branch of industry and occupational position. An attempt was also made to find out how many had changed their occupations or had discontinued to earn an income by reason of asthma and also the incidence of income-earners among the married women living with their husbands.

### Distribution according to branch of industry

Table 40 shows the distribution into

different branches of industry of the economically active men and women in this series of cases, compared with the general population of the same district according to the population census 1960. The figures given apply to the 1st November 1960.

Economically active persons are — according to the population census 1960 — defined as those who during the so-called population census week, carried out income yielding work comprising at least half the normal working hours. Also regarded as economically active were those persons who had income-yielding work but because of temporary discontinuation — e.g. vacation illness, military repetition exercises, temporary unemployment etc. — were not working during the population census week. The discontinuation was regarded as temporary if the duration of the interval was not more than 4 months. The same criteria applied essentially to the patients in the asthma series.

The total number of inhabitants in the collection area was about 95,000. The number of persons who were born during the period 1893–1932 was approximately 35,000. Of these 81.9% lived in the urban and 18.1% in the rural district. The corresponding figures for the asthma series were 81.1% and 18.9% respectively.

Branch of Industry	Male			Female		
	Asthma series 1960		General population the district of the asthma series 1960 /	Asthma series 1960		General population the district of the asthma series 1960
	No	%		No	%	
Agriculture forestry work etc.	6	19.4	12.5			2
Manufacturing and handicraft trade	8	25.8	31.2	4	17.4	10.3
Building trades	3	9.7	15.6			1.1
Commerce	2	6.5	11.7	3	13.0	10.3
Transport and communication	2	6.5	6.0	1	4.5	2.1
General administration	10	32.3	22.6	13	63.2	34.1

Table 40. Economically active males and females in the asthma series classified according to branch of industry compared with the corresponding figures for the general population in the same district 1960.

ered that the change had had a favorable effect on his asthma.

Case No. 13 L. E. Mann, born 1925. Onset of asthma 1932. In 1930 the patient got a job working as gardener watering the flowers etc. prevalent symptoms of asthma. He became retrained as a machanic in a two-year course at an engineering college and had worked such since 1932. He considered that the change of occupation had had a favorable effect on the course of the disease.

Case No. 14 L. G. Womack, born 1921. Onset of asthma 1932. In 1933 she discontinued farm work, which had mainly consisted of feeding cattle, and underwent training to become a children nurse at which she worked from 1933 on. She considered that the change of occupation had had a favorable effect on the course of her asthma.

Case No. 15 H. E. Ma, born 1923. Onset of asthma 1925. In 1917 he left his work and became a workshop balancer where several of the tasks he had to perform provoked asthma. He considered that the change had had a favorable effect on the course of the disease.

Case No. 21 S. L. Mann, born 1921. Onset of asthma 1930. In 1930 he changed his occupation from workshop carpenter to a laborer because of the dust in his former place of work. He considered that the change had improved the course of his asthma.

Case No. 22 P. O. Ma, born 1914. Onset of asthma 1916. The patient had worked previously in the factory but on account of the profuse dust he changed over in 1933 to work as a plaster. He considered that the change of occupation had effected his asthma favorably.

Case No. 46 J. V. Womack, born 1912. Onset of asthma 1916. In 1933 the patient discontinued his workshop assistant job and took this type of work (different) on account of her asthma and since 1933 she had worked as a factory cleaner. She did not consider that the change had had an effect on the course of the asthma.

Case No. 47 T. H. Mann, born 1912. Onset of asthma 1916. Since the patient was unable to tolerate the threshing dust among other things he changed over from his work in the

factory work. He considered that the change had had a favorable effect on the course of his asthma.

Case No. 60 J. O. H. M., born 1903. Onset of asthma 1911. In 1919 he discontinued his work in the factory since his asthma was worsened by much of the work that he had to perform. He changed over to a more types of work in the factory permanent position, but worked mostly as a salesman. In 1931 he received his pension and since that year in 1939 he tried working in a saw mill for a short time but had to finish when he found that this made his asthma worse. In 1939 he underwent a course of rehabilitation workshop after which he obtained employment as a saleswoman. This occupation he resumed. The patient did not consider that his changes of occupation had had any effect on the course of the asthma.

Case No. 61 L. E. W. Mann, born 1904. Onset of asthma 1917. In 1926 she changed her occupation from work to hospital orderly where she was unable to tolerate the smell of coughing. She did not consider that the change had had an effect on the course of her asthma.

Case No. 78 F. O. Mann, born 1902. Onset of asthma 1926. In 1916 he changed over from a railway to forestry work, since he was unable to tolerate the dust. He considered that the change had effected his asthma favorably.

Case No. 79 S. S. Mann, born 1902. Onset of asthma 1912. In 1931 he partly discontinued his work as a salesman, working only periods which as required his asthma was not so severe. He continued with this work until 1937 when he changed over to part time office work, which he was able to keep up for short periods off and on. His asthma, however, never permitted any permanent employment. The patient did not consider that the change of work had had any effect on the course of the disease.

Case No. 80 F. A. Mann, born 1901. Onset of asthma 1910. In 1936 he left his job in the saw yard and obtained work as a caretaker in block of flats. The reason for the change was partly that the former work involved considerable physical strain and also that the dust affected his asthma. He considered that

tendency to move from work involving physical strain to the more sedentary types of occupation. The demands of the different occupations on physical strain were assessed according to the norms established by Smårs and Berfenstam (1961). These authors distinguished between eight groups, in which the demand on physical strain decreased with increasing group numbers; these were combined to form four groups in the present study. Only 1 of the 15 men changed over to a heavier occupation while 8 changed to lighter and in 6 cases the working conditions were unchanged in this respect. The only employer before the change remained as such afterwards while one of the employees became an employer on changing his occupation.

The women included in this analysis were only few but the tendencies shown with regard to occupational changes appeared to agree with those of the men.

Some brief case-histories of those patients who changed their occupations by reason of asthma will be given below. The descriptions include the year of onset of the asthma and the type of work performed by the patient before and after his change of occupation. Also mentioned are the factors in the original work that were considered to be unsuitable with regard to the asthma and the patient's personal opinion as to whether or not the change of work had had a favourable influence on the course of the disease.

Case No 4. A. L. Man, born 1929. Onset of asthma 1936. In 1939 he discontinued work as a salesman for a leather and skin firm, since the skin provoked attacks of asthma. Obtained employment as a metal worker after attending a re-training course. The patient considered that the change had had a favourable effect on the course of the disease.

Case No 11. M. J. Man, born 1926. Onset of asthma 1942. Left farm work in 1945 and became a lorry driver. Since the contact with cattle provoked asthmatic attacks, he considered

	Sex	No. who changed	Average age at first change, yrs.	Branch of industry							No. employers	No. employees	Groups according to physical strain of the occupation			
				Agriculture forestry etc.	Manufacturing and handicraft trade	Building trades	Commerce	Transport and communication	General administration	1			2	3	4	
Before first change	M	13	38	1	4	1	1	1	1	14	11	1	2	1		
After first change	M			1	0	2	1	1	4	2	13	1	4	3	4	
Before first change	F	1	44	1	1		1		1	4		2	1			
After first change	F				1				2	4			2			

Table 50. Patients who changed their occupation because of bronchial asthma, all attributed according to branch of industry before and after the first change of occupation. Group according to physical strain of occupation based on those established by Smårs and Berfenstam (1961).

(7.3 %) of the men were included in both analyses, i.e. both in that of the changes of occupation and in that of the patients who had discontinued to earn an income. Of these three men there was one who after an interval of about two years returned to work.

The study with regard to the discontinuation of work showed — as in the case of changes of occupation — that several men gave up agricultural and forestry work. The same also applied to the manufacturing and handicraft trades, while the general administration branch lost relatively fewer workers. Of the men who had permanently stopped working (10 altogether) one was an employer and the rest employees. There was a clear majority (8 out of 10) in the two groups in

which the greatest degree of physical strain was demanded, and there were none in the group where the other extreme applied. The average age of the men at permanent discontinuation of income-earning work was 53 years.

With regard to the women, 10 (28 %) had permanently discontinued earning their living because of asthma. This percentage figure was based on the 36 women who at some time had had steady income-yielding work comprising at least  $\frac{1}{2}$  the usual working hours. The average age of the women at permanent discontinuation of income-earning work was 48 years.

Apart from these 10 women, a further four had discontinued earning an income but their work had comprised fewer than half time hours. The

Sex	No.	Average age at first discontinuation	Branch of industry which the patient left				Before second operation		Groups according to physical strain in that occupation which the patient left			
			Agriculture, forestry and fishery work	Manufacturing and handicraft trades	Commerce	General administration	No. of employers	No. of employees				
									1	2	3	4
Discontinued income-earning	M	10	33	3	1	3	1	9	4	4	2	
Discontinued income-earning, but began again		2	40	1		1		2	1			1
Discontinued income-earning	F	10	48 (47)	0 (2)		3 (9) 7 (3)	1 (1)	9 (8)		2 (2)	6 (1)	0 (1)
Discontinued income-earning, but began again		4	30		2	2		4		1	1	2

Table 81. Discontinuation of income-earning because of asthma.

These patients completely or by at least two-thirds discontinued income-earning, this being comprised at least half the usual working hours. The main reason for the discontinuation was bronchial asthma. The figures in parentheses refer to those whose occupation comprised fewer than half-time hours. The T also shows those who after an interval of more than one year again began earning, with the same work, before different, but the two comprising at least half the usual hours. Groups according to physical strain of occupation based on those established by Sjöström and Berntson (1961).



the course of the asthma had improved since he changed his occupation.

Case No 81 A. L. Man, born 1901 Onset of asthma 1930. In 1936 he changed from pipe-laying to general labouring. The reason was to avoid working in draughty places. The change was considered to have had no effect on the course of the asthma.

Case No 103 V. H. Man, born 1897 Onset of asthma 1936. In 1945 he changed over from driving a milk lorry to office work — both positions at the Milk Distribution Centre. The reason for the change was that the patient was unable to manage the relatively heavy work as a lorry driver. The change appeared to have no effect on the course of the asthma.

Case No 113. E. W. Woman, born 1890. Onset of asthma 1947. During the same year she had to give up her work in a bakery (mainly as a bread packer). She was unable to work for almost a year because of severe asthma but then obtained employment in a brewery. The patient's old place of employment was at that time unable to provide work for her and this was the main reason for the change. She did not consider that the change of occupation had had any influence on the course of the disease.

Case No 116. E. K. Man, born 1893 Onset of asthma 1919. Received an invalid pension in 1951 on account of asthma. In 1950 he gave up working as a combined farm labourer and builder and in the same year began part time work as an odd job man. He considered that the change had had a favourable effect on the course of the asthma since the former work had involved too much physical strain.

Case No 125 H. A. Man born 1893 Onset of asthma 1910. At the end of 1943 he left his work as a book binder and became a dilettante — self-employed. He was unable to tolerate the book dust in his old place of employment. He considered that the asthma if anything had deteriorated since the change. He chose to change to the position of employer mainly because of his asthma.

One male patient (case No 72) born 1903, whose asthma was first manifested in 1914 was excluded from Table 50. Because his asthma was provoked by contact with cattle he sold all the cattle on his farm in

1952 and also part of the land, since the asthma had reduced his working capacity. As, however, the patient still maintained part of the farm and continued to earn a living as a farmer he was not included in the table. The course of disease was affected favourably by the measures taken by the patient in 1952.

As shown by the case histories, if this latter patient was not included, 11 out of the 19 who had changed their occupations on account of asthma considered that this change had a favourable effect on the course of the disease. Seven considered that there was no effect, and one patient thought that his asthma had deteriorated somewhat by reason of his change of occupation.

#### *Discontinuation of income yielding work*

Table 51 shows the number of patients who mainly because of asthma had completely or by at least 2/3 permanently discontinued income yielding work which had comprised at least half the usual working hours. The table also gives the number of patients who similarly discontinued work — also of at least half the usual hours — but began again after an interval of more than one year.

As seen in the table 10 (24 %) of the men discontinued permanently while 2 (5 %) discontinued but after an interval of more than one year became economically active again. Taking into consideration the fact that a further 15 (38 %) of the men in the material found it necessary to change their occupations, it is seen that a clear majority of the men were markedly affected by the disease in this respect. It should be noted, however, that 7

(7.5 %) of the men were included in both analyses, i. e. both in that of the changes of occupation and in that of the patients who had discontinued to earn an income. Of these three men there was one who after an interval of about two years returned to work.

The study with regard to the discontinuation of work showed — as in the case of changes of occupation — that several men gave up agricultural and forestry work. The same also applied to the manufacturing and handicraft trades, while the general administration branch lost relatively fewer workers. Of the men who had permanently stopped working (10 altogether) one was an employer and the rest employees. There was a clear majority 3 out of 10 in the two groups in

which the greatest degree of physical strain was demanded and there were none in the group where the other extreme applied. The average age of the men at permanent discontinuation of income-earning work was 51 years.

With regard to the women, 10 (28 %) had permanently discontinued earning their living because of asthma. This percentage figure was based on the 36 women who at some time had had steady income-yielding work comprising at least  $\frac{1}{3}$  the usual working hours. The average age of the women at permanent discontinuation of income-earning work was 48 years.

Apart from these 10 women a further four had discontinued earning an income but their work had comprised fewer than half time hours. The

	Sex	No.	Average age at first discontinuation	Branch of industry which the patient left				Before discontinuation		Occupations according to physical strain in their occupation within the period left				
				Agriculture Forestry work etc.	Manufacturing and handicraft trades	Commerce	General administration	No. of employees	No. of employer	1	2	3	4	
Discontinued income-earning because of asthma	M	10	53	2	1	3		1	0		4	4	2	
Discontinued income-earning but began again	M	3	40	1			3			2			1	
Discontinued income-earning but began again	F	10 (16 of 47)		0 (0)		3 (0)	7 (2)	1 (1)	0 (3)			2 (2)	3 (1)	0 (1)
Discontinued income-earning but began again	F	4	50		2		2			4		5	2	2

Table 61. Discontinuation of income-earning because of asthma.

These patients completely or by at least two-thirds discontinued income-earning. This has been compared at least half the usual working hours. The main reason for the discontinuation was bronchial asthma. The figures in parentheses refer to those whose occupation comprised fewer than half-time hours. The Table also shows those who after an interval of more than one year again began earning, with the same work before or different, but this too excepted on those established by Späth's test (discontinuation of work).

figures given in parenthesis in the table apply to these women. They are not included in the ordinary figures.

The table also shows that four other women had similarly ceased working because of bronchial asthma but they had later returned to the same or a different occupation after an interval of more than one year.

The table includes (in parenthesis) two farmers' wives who stated that the family farming property had had to be sold for the main reason that the wives were unable to take part in the outdoor work because of asthma. In one of these cases the husband was a semi-invalid and in both cases the husbands and wives were about 60 years old. It was rather difficult, however, to determine the predominant reason for the disposal of the farms. These women are included in the table among those whose work was assessed as comprising fewer than half of the usual working hours.

#### *Income yielding work among married women living with their husbands*

Table 52 shows the number of economically active and inactive married

women living with their husbands, in both the asthma series and the Uppsala population, distributed according to age. As may be seen in the table, there is almost exact agreement between the two entire series with regard to the incidence of persons with income yielding work. In two of the different age groups there is a somewhat greater difference between the asthma series and the Uppsala population but regarded separately these two groups are much too small to permit any true assessment. The figures for the Uppsala population were calculated from the population census of the 1st November 1900.

To summarize it may be said that the distribution of the economically active patients in the asthma material into different branches of industry did not differ significantly from that in the total population. A study of the

Age, years	30—39			40—49			50—59			60—69			70—79		
	Asthma series		Corresponding general population Uppsala	Asthma series		Corresponding general population Uppsala	Asthma series		Corresponding general population Uppsala	Asthma series		Corresponding general population Uppsala	Asthma series		Corresponding general population Uppsala
	No.	%		No.	%		No.	%		No.	%		No.	%	
Economically active	2	72.2	32.2	8	61.5	36.0	4	30.8	28.2	1	6.3	8.0	1	20.4	29.0
Economically inactive	7	77.8	67.8	5	38.5	64.0	9	69.2	71.8	15	93	91.1	30	79.6	71.0
Total	9	100.0	100.0	13	100.0	100.0	13	100.0	100.0	16	100.0	100.0	31	100.0	100.0

Table 52. Economically active and inactive non-invalid married women in the asthma series married and living with their husbands distributed by age compared with the corresponding figures for the general population of Uppsala 1900.

By economically active women is meant here those with income-yielding work comprising at least half the ordinary working hours.

distribution into occupational positions showed that a relatively great number of the asthma patients in relation to the Uppsala population were to be found in the group of self-employers.

About every 3rd man and every 10th woman changed their occupation on at least one occasion on account of asthma. Approximately every 4th man and woman discontinued income-yielding work permanently because of asthma. No significant difference compared with the Uppsala population was shown with regard to the incidence of persons with income yielding work among the married women in the asthma series, living with their husbands.

### Investigation of the income level of the economically active patients

A study was made of the income level in December 1963 of the non-invalid pensioned patients born in 1897 or later using as a basis the sickness benefit classes to which they were assigned by the General Sickness Insurance. 1963 was chosen because this was the first year in which not only income earned as an employee but also income obtained by different forms of self-employment was taken into consideration at the General Sickness Insurance, where there is a relationship between income level and the sickness benefit class to which a person is assigned. Corresponding to each of the sickness benefit classes there is a certain amount of daily sickness benefit to which a person is entitled. The amount of this sickness benefit in different income-level groups is shown in Table 53. The table also shows that

the income scale is only differentiated up to 21 000 Swedish crowns of annual income.

Information was obtained as to the amount of sickness benefit to which each of the patients in the asthma series was entitled. For control purposes information was also obtained with regard to the corresponding amount of sickness benefit for the 14 persons of the same sex as the patient — entered in the register of the General Sickness Insurance immediately before and the 14 persons entered immediately after the asthma patient concerned. This register is made up according to the date of birth of the insured person. The sickness benefit of these four persons was added together and the figure obtained divided by 4. The final figure was then compared with the benefit to which the asthma patient was entitled. For each asthma patient a control figure was thus obtained. Since invalid pensioners were excluded from the asthma material the same were also excluded from this control material.

By the procedure described above it was possible to obtain a mean group figure both for the men and for the women of the asthma and the control materials. For the men in the asthma

Annual earned income		Sickness benefit
Sw Cr	Sw Cr	
1800	2800	5
3000	3100	6
3400	4200	7
4200	5000	8
5000	5600	9
5900	6300	10
6900	8400	12
8100	10200	14
10200	12000	16
12000	14000	18
14000	16000	20
16000	18000	22
19000	21000	25
21000	—	26

Table 53. The daily sickness benefit from the General Sickness Insurance in different income levels.

figures given in parentheses in the table apply to these women. They are not included in the ordinary figures.

The table also shows that four other women had *similarity ceased working* because of bronchial asthma but they had later returned to the same or a different occupation after an interval of more than one year.

The table includes (in parentheses) two farmers' wives who stated that the family farming property had had to be sold for the main reason that the wives were unable to take part in the outdoor work because of asthma. In one of these cases the husband was a semi-invalid and in both cases the husband and wives were about 60 years old. It was rather difficult, however, to determine the predominant reason for the disposal of the farms. These women are included in the table among those whose work was assessed as comprising fewer than half of the usual working hours.

#### *Income yielding work among married women living with their husbands*

Table 52 shows the number of economically active and inactive married

women, living with their husbands, in both the asthma series and the Uppsala population distributed according to age. As may be seen in the table there is almost exact agreement between the two entire series with regard to the incidence of persons with income-yielding work. In two of the different age groups there is a somewhat greater difference between the asthma series and the Uppsala population, but regarded separately these two groups are much too small to permit any true assessment. The figures for the Uppsala population were calculated from the population census of the 1st November 1960.

To summarize it may be said that the distribution of the economically active patients in the asthma material into different branches of industry did not differ significantly from that in the total population. A study of the

Age years	30-39						40-49						50-59						60-69						70-79					
	Asthma series		Corresponding general population Uppsala				Asthma series		Corresponding general population Uppsala				Asthma series		Corresponding general population Uppsala				Asthma series		Corresponding general population Uppsala				Asthma series		Corresponding general population Uppsala			
	No.	%	%	No.	%	%	No.	%	%	No.	%	%	No.	%	%	No.	%	%	No.	%	%	No.	%	%	No.	%	%			
Economically active	22	2.2	33.2	8	8.1	86.0	4	30.8	28.2	1	6.3	8.9	13	20.4	20.0															
Economically inactive	8	0.8	67.8	2	38.5	61.0	0	60.3	1.8	1	83	91.1	26	70.6	1.0															
Total	30	100.0	100.0	10	100.0	100.0	4	100.0	100.0	2	100.0	100.0	39	100.0	100.0															

Table 52. Economically active and inactive married women in the asthma series and living with their husbands, distributed by age compared with the corresponding figures of the general population of Uppsala 1960.

By "economically active women" is meant here those with income-yielding work comprising (at least) the ordinary working hours.

distribution into occupational positions showed that a relatively great number of the asthma patients in relation to the Uppsala population were to be found in the group of self-employers.

About every 3rd man and every 10th woman changed their occupation on at least one occasion on account of asthma. Approximately every 4th man and woman discontinued income-yielding work permanently because of asthma. No significant difference compared with the Uppsala population was shown with regard to the incidence of persons with income yielding work among the married women in the asthma series, living with their husbands.

### Investigation of the income level of the economically active patients

A study was made of the income level in December 1963 of the non-fatal pensioned patients born in 1897 or later using as a basis the sickness benefit classes to which they were assigned by the General Sickness Insurance 1963 was chosen because this was the first year in which not only income earned as an employee but also income obtained by different forms of self-employment was taken into consideration at the General Sickness Insurance, where there is a relationship between income level and the sickness benefit class to which a person is assigned. Corresponding to each of the sickness benefit classes there is a certain amount of daily sickness benefit (which a person is entitled to). The amount of this sickness benefit in different income-level groups is shown in Table 33. The table also shows that

the income scale is only differentiated up to 21 000 Swedish crowns of annual income.

Information is obtained as to the amount of sickness benefit to which each of the patients in the asthma series was entitled. For control purposes information was also obtained with regard to the corresponding amount of sickness benefit for the two persons of the same sex, the patient nearest to the register of the General Sickness Insurance immediately before and the 10 persons entered immediately after the asthma patient concerned. This register is made up according to the date of birth of the insured person. The sickness benefit for these four persons are added together and the figure obtained divided by four. The final figure was then compared with the benefit to which the asthma patients are entitled. For each asthma patient his own figure and a control figure were thus obtained where the last two figures were excluded from the asthma material they were also excluded from this control material.

By the procedure described above it was possible to obtain a mean group figure both for the men and for the women of the asthma and the control materials. For the men in the asthma

Annual earned income		Sickness benefit
men had % Cr	but not % Cr	
1800	2000	8
2000	2100	11
2100	2200	7
2200	2300	8
2400	2400	9
2600	2600	10
2800	2800	12
3000	3000	14
3200	3200	16
3400	3400	18
3600	3600	20
3800	3800	22
4000	4000	24
4200	—	26

Table 33. The daily sickness benefit from the General Sickness Insurance in different income levels.

figures given in parenthesis in the table apply to these women. They are not included in the ordinary figures.

The table also shows that four other women had similarly ceased working because of bronchial asthma but they had later returned to the same or a different occupation after an interval of more than one year.

The table includes (in parenthesis) two farmers' wives who stated that the family farming property had had to be sold for the main reason that the wives were unable to take part in the outdoor work because of asthma. In one of these cases the husband was a semi-invalid and in both cases the husbands and wives were about 60 years old. It was rather difficult, however, to determine the predominant reason for the disposal of the farms. These women are included in the table among those whose work was assessed as comprising fewer than half of the usual working hours.

#### *Income yielding work among married women living with their husbands*

Table 52 shows the number of economically active and inactive married

women, living with their husbands, in both the asthma series and the Uppsala population distributed according to age. As may be seen in the table, there is almost exact agreement between the two entire series with regard to the incidence of persons with income yielding work. In two of the different age groups there is a somewhat greater difference between the asthma series and the Uppsala population but regarded separately these two groups are much too small to permit any true assessment. The figures for the Uppsala population were calculated from the population census of the 1st November 1960.

To summarize it may be said that the distribution of the economically active patients in the asthma material into different branches of industry did not differ significantly from that in the total population. A study of the

Age years

30—39			40—49			50—59			60—69			70—79		
No.	Asthma series		No.	Corresponding general population of Uppsala		No.	Corresponding general population of Uppsala		No.	Corresponding general population of Uppsala		No.	Corresponding general population of Uppsala	
	%	%		%	%		%	%		%	%		%	%

Economically  
active  
Economically  
inactive  
Total

2	22.2	32.2	8	61.5	36.0	4	30.8	28.2	1	6.3	8.9	15	70.4	29.0
	77.8	67.8	5	38.5	64.0	9	69.2	71.8	13	93.7	91.1	30	70.6	1.0
10	100.0	100.0	13	100.0	100.0	13	100.0	100.0	18	100.0	100.0	1	100.0	100.0

Table 52. Economically active and inactive married women living with their husbands distributed by age compared with the corresponding figures for the general population of Uppsala 1960.

By economically active women I mean here those with income-yielding work comprising at least half of the ordinary working hours.

corresponding figures for December 1960 and December 1963 were 46 % and 50 % respectively. The tendency in these men in the asthma series towards an improvement in their level of income during the period studied, in comparison with the control material, did not reach a degree of significance.

Out of all the employed men in the asthma series in 1935 one was excluded from this study because he received an invalid pension after that year.

To summarize the mean income of the men in the asthma series born in 189 or later and who were not receiving an invalid pension, did not differ significantly from the expected figure. The mean income of the economically active women in the asthma series, on the other hand, was lower than the expected figure. The incidence of economically active women in the asthma material was in close agreement with that in the total population.

The income development of the economically active employed men in the asthma series during the period 1935—63 did not appear to differ significantly from that of the total population.

### Housing standard and size

#### Housing standard

It is often said that there is a certain

degree of relationship between poor housing standards and asthma. The study of the standard of housing of these patients was therefore of interest not only as a measure of their social adaptation but also in seeking to obtain an idea of the extent to which such a relationship might apply to the individuals in the asthma series. In an attempt to determine the degree to which the apartments of these patients could be said to fulfil reasonable demands of quality and size the housing standard of each patient was compared with that of the so-called median person living in the same district and belonging to the same civil state and age group.

In classifying the apartments from a point of view of standard, the principles shown in Table 54 were used, the standard of the apartment decreasing with increasing group numbers. An apartment for example, fitting all reasonable requirements of modern standard, was assigned to group 1 while its opposite was placed in group 7. It was found that 70 % of the patients lived in apartments of standard degree 1, 5 % of degree 2, 8 % of degree 3, 6 % of degree 4, 1 % of degree 5, 8 % of degree 6 and 4 % of degree 7.

with water and drain						
with w. and central heating				with w.	without w.c.	
with or without bathroom or shower		without w.c. bathroom or shower	with w. without central heating	with central heating	without central heating	without water and/or drain
with modern cooker and refrigerator	without modern cooker and/or refrigerator					
standard-gr 1	st-gr 2	st-gr 3	1-gr 4	1-gr 5	st-gr 6	st-gr 7

Table 54. Classification of housing conditions into standard groups.



series this was 19.9 Swedish crowns, and for the men in the control material 20.0 Swedish crowns. 13 of the men in the asthma series attained a higher figure than the mean figure for the men in the control material with whom each of the patients was compared. 13 attained a lower figure and 2 showed the same figure.

In the asthma series there were 53 women who were born in 1897 or later and who were not receiving invalid pensions. Of these 24 (45.3 %) were registered at the General Sickness Insurance as having an earned income exceeding 1800 Swedish crowns per year. These patients were entitled to a mean daily sickness benefit payment of 12.0 Swedish crowns. As mentioned above for each of these 53 women there were four control persons. Of these 212 90 (42.5 %) were registered as having an earned income, and they were entitled to a mean daily sickness benefit payment of 15.2 Swedish crowns. Thus the sickness benefit to which the women in the asthma series who were registered as having an earned income, were entitled was on an average lower than the corresponding benefit in the control material. The difference between the asthma series and the control material was significant ( ). On the other hand there was no significant difference with regard to the percentage number of women with an estimated annual income of at least 1800 Swedish crowns.

When the male self-employed were excluded from both the asthma series and the control material a mean figure of 21.7 Swedish crowns was obtained for the asthma series, while the

corresponding figure for the control material was 21.1 Swedish crowns. With regard to the men in the asthma series, nine attained a higher figure than the mean figure for the men in the control material with whom each of the patients were compared, ten showed a lower figure and two the same figure.

To obtain an idea of the income development during the period 1955-1963 for the men in the asthma series born in 1897 or later who in 1963 were not receiving an invalid pension, and who were not self-employed during the period 1955-1963 the following method was used. (Before the year 1963 self-employed were not registered by the General Sickness Insurance according to their income levels and could therefore not be included.)

The men in the asthma series were divided into groups according to age and the district in which they lived and the men in the total population were grouped in a similar way. Each man in the asthma series was then ranked as described on page 31 in relation to the median person in the total population, each patient being given a percentage figure which indicated his income level in relation to that of the median person, which was determined as 50 %. This method was used for comparing the mean income level in the asthma series and in the control material for December 1955 and for December 1963. The procedure was principally the same when a comparison was made for December 1963. The patients in the asthma series were divided into age groups. Each patient was then ranked in relation to the control material for December 1963, obtained from the General Sickness Insurance still with the exclusion of the self-employed.

It was found that for December 1955 the men in the asthma series reached a mean ranking figure of 40 %. The

corresponding figures for December 1960 and December 1963 were 46 % and 50 % respectively. The tendency in these men in the asthma series towards an improvement in their level of income during the period studied, in comparison with the control material, did not reach a degree of significance.

Out of all the employed men in the asthma series in 1955 one was excluded from this study because he received an invalid pension after that year.

To summarize the mean income of the men in the asthma series born in 1897 or later and who were not receiving an invalid pension, did not differ significantly from the expected figure. The mean income of the economically active women in the asthma series, on the other hand, was lower than the expected figure. The incidence of economically active women in the asthma material was in close agreement with that in the total population.

The income development of the economically active employed men in the asthma series during the period 1955-63 did not appear to differ significantly from that of the total population.

### Housing standard and size

#### Housing standard

It is often said that there is a certain

degree of relationship between poor housing standards and asthma. The study of the standard of housing of these patients was therefore of interest not only as a measure of their social adaptation but also in seeking to obtain an idea of the extent to which such a relationship might apply to the individuals in the asthma series. In an attempt to determine the degree to which the apartments of these patients could be said to fulfill reasonable demands of quality and size the housing standard of each patient was compared with that of the so-called median person living in the same district and belonging to the same civil state and age group.

In classifying the apartments from a point of view of standard, the principles shown in Table 84 were used, the standard of the apartment decreasing with increasing group numbers. An apartment for example fulfilling all reasonable requirements of modern standard, was assigned to group 1 while its opposite was placed in group 7. It was found that 70 % of the patients lived in apartments of standard degree 1, 5 % of degree 2, 8 % of degree 3, 6 % of degree 4, 1 % of degree 5, 6 % of degree 6 and 2 % of degree 7.

with w. ltr and drains						
with w. and central heating			with w.c., w. floor central heating	without w.c.		
with own bathroom or shower		w. toilet on bathroom or shower		ith central heating	ithout central heating	withou t w.c. and/or drain
with modern cooker and refrigerator	without modern cooker and/or refrigerator					
standard-gr. 1	st-gr. 2	st-gr. 3	1-gr. 4	st-gr. 5	st-gr. 6	1-gr. 7

Table 84. Classification of housing conditions into standard groups.

series this was 19.0 Swedish crowns, and for the men in the control material 20.0 Swedish crowns. 13 of the men in the asthma series attained a higher figure than the mean figure for the men in the control material with whom each of the patients was compared. 13 attained a lower figure and 2 showed the same figure.

In the asthma series there were 53 women who were born in 1897 or later and who were not receiving invalid pensions. Of these, 24 (45.3 %) were registered at the General Sickness Insurance as having an earned income exceeding 1800 Swedish crowns per year. These patients were entitled to a mean daily sickness benefit payment of 12.0 Swedish crowns. As mentioned above, for each of these 53 women there were four control persons. Of these 212.00 (42.5 %) were registered as having an earned income, and they were entitled to a mean daily sickness benefit payment of 16.2 Swedish crowns. Thus the sickness benefit to which the women in the asthma series, who were registered as having an earned income, were entitled, was on an average lower than the corresponding benefit in the control material. The difference between the asthma series and the control material was significant (\*). On the other hand there was no significant difference with regard to the percentage number of women with an estimated annual income of at least 1800 Swedish crowns.

When the male self-employed were excluded from both the asthma series and the control material, a mean figure of 21.7 Swedish crowns was obtained for the asthma series, while the

corresponding figure for the control material was 21.1 Swedish crowns. With regard to the men in the asthma series, nine attained a higher figure than the mean figure for the men in the control material with whom each of the patients were compared, ten showed a lower figure and two the same figure.

To obtain an idea of the income development during the period 1935—1963 for the men in the asthma series born in 1897 or later who in 1963 were not receiving an invalid pension and who were not self-employed during the period 1935—1963 the following method was used. (Before the year 1963 self-employed were not registered by the General Sickness Insurance according to their income levels and could therefore not be included.)

The men in the asthma series were divided into groups according to age and the district in which they lived, and the men in the total Uppsala population were grouped in a similar way. Each man in the asthma series was then ranked as described on page 91 in relation to the median person in the total population, each patient being given a percentile figure which indicated his income level relative to that of the median person, which was determined as 50 %. This method was used for comparing the mean income level in the asthma series and the control material for December 1935 and for December 1960. The procedure was principally the same when comparison was made for December 1963. The patients in the asthma series were divided into age groups. Each patient was then ranked in relation to the control material for December 1963. Excluded from the General Sickness Insurance, still with the status of the self-employed.

It was found that for December 1960 the men in the asthma series reached a mean ranking figure of 40 %. The

corresponding figures for December 1900 and December 1903 were 46 % and 50 % respectively. The tendency in these men in the asthma series towards an improvement in their level of income during the period studied, in comparison with the control material, did not reach a degree of significance.

Out of all the employed men in the asthma series in 1935 one was excluded from this study because he received an invalid pension after that year.

To summarize the mean income of the men in the asthma series born in 1897 or later and who were not receiving an invalid pension, did not differ significantly from the expected figure. The mean income of the economically active women in the asthma series, on the other hand, was lower than the expected figure. The incidence of economically active women in the asthma material was in close agreement with that in the total population.

The income development of the economically active employed men in the asthma series during the period 1936-53 did not appear to differ significantly from that of the total population.

### Housing standard and size

#### Housing standard

It is often said that there is a certain

degree of relationship between poor housing standards and asthma. The study of the standard of housing of these patients was therefore of interest not only as a measure of their social adaptation but also in seeking to obtain an idea of the extent to which such a relationship might apply to the individuals in the asthma series. In an attempt to determine the degree to which the apartments of these patients could be said to fulfill reasonable demands of quality and size the housing standard of each patient was compared with that of the so-called median person living in the same district and belonging to the same civil state and age group.

In classifying the apartments from a point of view of standard, the principles shown in Table 54 were used, the standard of the apartment decreasing with increasing group numbers. An apartment for example, fulfilling all reasonable requirements of modern standard was assigned to group 1 while its opposite was placed in group 7. It was found that 70 % of the patients lived in apartments of standard degree 1, 3 % of degree 2, 8 % of degree 3, 0 % of degree 4, 1 % of degree 5, 8 % of degree 6 and 2 % of degree 7.

with water and drain						
with water and central heating			without w.c.			
with own bathroom or shower		without own bathroom or shower	with w.c. without central heating		with central heating	without central heating
with modern cooker and refrigerator	without modern cooker and/or refrigerator		without central heating		without water and/or drain	
standard gr 1	sl-gr 2	L-gr 3	1-gr 4	sl-gr 5	L-gr 6	L-gr 7

Table 54 Classification of housing conditions into standard groups.

Table 56 shows the housing standard in the asthma series each patient being compared with the median person in the total population according to the so-called "ranking method" described on page 31. The table distinguishes between heads of the household belonging to the asthma series i.e. men and unmarried or divorced women and widows and heads of the household who belong to the rest of the population i.e. men who are married and live with their wives who belong to the asthma series. As seen in the table the asthma series as a whole compared well with the total Uppsala population. The median control person in the total population is placed at figure 60. None of the groups shown in the table differed significantly from the expected figure of 60.

As seen in Table 56 the mean ranking figure for the housing standard for the total asthma series was 53.6. Those living in the rural districts had a somewhat higher figure and those in the urban districts somewhat lower. When the series was divided into two groups, one in which the patients themselves were heads of the household and one in which the patient's husband was the head of the household a mean figure of 50.1 was obtained for the former and 56.3 for the latter.

For the invalid pensioners included in the series the mean figure was 52.4 and when these were divided into two groups according to whether or not the head of the household was part of the asthma series the figures obtained were 51.2 and 54.0 respectively. The corres-

Groups according to age yrs.	Groups according to so-called ranking figures					Mean ranking figure
	0-20	21-40	41-60	61-80	81-100	
30-34	I	2	2	2		43.8
	II	1	3			48
35-39	I	1	2	2		51.0
	II		4			54.4
40-44	I		1			58.1
	II		4	1		61.6
45-49	I	3	2			40.3
	II		2	1		65.9
50-54	I		1	1	1	66.3
	II	1		9		53.9
55-59	I	2	2	13	1	52.9
	II	2	3	11	2	57.7
60-64	I	3	1	4		50.0
	II	2		10		51

Table 56. Standard of housing conditions 1960 according to age for each of the individual in the asthma series expressed in accordance with the so-called ranking method (page 31) where each individual in the asthma series is compared with the so-called median person within the same age-sex and civil status group in the total population (urban and rural districts population).

I = head of household, included in the asthma series.  
 II = head of household, living with wife who is included in the asthma series.

pending figures for the non invalid pensioners in the series were 51.2 and 55.8 respectively)

When the patients in the total series were divided into groups according to the mean annual number of sickness points during the period 1937-61 it was found that those who during this period belonged to group 3, i.e. those with the most symptoms (estimated mean annual sickness points of 100 or more) had a mean ranking figure

of 51.4. Within this group there was a not unessential difference between the group in which the head of the household was included in the asthma series which group showed a figure of 43.2, and that comprising married women living with their husbands where the husbands were head of the household. This latter category showed a figure of 64.2. For those on the other hand, who during the same period belonged to the group with the least symptoms accord

Category	No.	Ranking figure	
		Standard	Size
Whole mal. asthma series	116	53.6	47.3
Town residents	91	53.9	45.3
Rural district residents	25	56.6	56.8
Widow and single and other women who are themselves head of the household	56	50.7	46.3
Married women living with their husbands, where the husband is head of the household	60	56.3	49.3
Invalid pensioners, total	21	52.4	37.0
Invalid pensioners who are themselves head of the household	13	51.2	26.1
Invalid pensioners, whose husband is head of the household	8	54.9	37.7
Non invalid pensioners, total	95	53.8	49.6
Non invalid pensioners who are themselves head of the household	42	51.3	
Non invalid pensioners, whose husband is head of the household	53	53.8	
Patients who during the period 1937-61 attained an annual mean sickness point figure of 100 or more	18	51.4	39.3
Those of these patients who are themselves head of the household	11	43.2	
Those of these patients whose husband is head of the household	7	64.2	
Patients who during the period 1937-61 attained annual mean sickness point figure of less than 50	60	54.8	50.6
Those of these patients who are themselves head of the household	31	53.7	
Those of these patients whose husbands are head of the household	29	53.7	
Patients who according to Table 3 belong to group A, i.e. up to the end of 1960 acquired fewer than 1000 sickness points	28	56.9	57.3
Those of these patients who are themselves head of the household	15	60.8	59.8
Those of these patients whose husband is head of the household	23	51.2	51.6
Patients who according to Table 3 belong to group B, i.e. up to the end of 1960 acquired 1000 but not 2000 sickness points	41	58.4	42.7
Those of these patients who are themselves head of the household	22	45.9	41.3
Those of these patients whose husband is head of the household	19	55.6	40.7
Patients who according to Table 3 belong to group C, i.e. up to the end of 1960 acquired at least 2000 sickness points	37	53.8	42.3
Those of these patients who are themselves head of the household	19	48.4	38.0
Those of these patients whose husbands are head of the household	18	60.6	46.8

Table 54 Mean ranking figures with regard to standard and size of patient for different groups of patient included in the material

Table 55 shows the housing standard in the asthma series each patient being compared with the median person in the total population according to the so called "ranking method" described on page 31. The table distinguishes between heads of the household belonging to the asthma series, i.e. men and unmarried or divorced women and widows, and heads of the household who belong to the rest of the population, i.e. men who are married and live with their wives who belong to the asthma series. As seen in the table, the asthma series as a whole compared well with the total Uppsala population. The median control person in the total population is placed at figure 50. None of the groups shown in the table differed significantly from the expected figure of 50.

As seen in Table 56 the mean ranking figure for the housing standard for the total asthma series was 53.6. Those living in the rural districts had a somewhat higher figure and those in the urban districts somewhat lower. When the series was divided into two groups, one in which the patients themselves were heads of the household and one in which the patient's husband was the head of the household, a mean figure of 59 was obtained for the former and 56.3 for the latter.

For the invalid pensioners included in the series the mean figure was 52.4 and when these were divided into two groups according to whether or not the head of the household was part of the asthma series the figures obtained were 51.2 and 54.0 respectively. The corre-

Groups according to age yrs	Groups according to so-called ranking figures					Mean ranking figure
	0-20	21-40	41-60	61-80	81-100	
18-31	1	2	2	2		53.6
32-45	11	1	2			46.8
46-59	1	1	2			52.0
60-74	11		4			54.1
75-89	1		1			59.7
90-100	11		4	1		61.0
10-20	1	3	2	2		49.3
21-30	11		2	1		64.8
31-40	1		1	4	1	66.3
41-50	11	1		9		52.0
51-60	1	2	1	13	1	54.8
61-70	11	2	1	13	2	56.0
71-80	1	3	1	4		51.0
81-90	11	2	2	10		52.1

Table 55. Standard of housing conditions 1960 according to age for each of the individuals in the asthma series expressed in accordance with the so-called ranking method (page 31) where each individual in the asthma series is compared with the so-called median person within the group of age and class to be compared in the total population (urban and rural district population).

I = be 1 of household included in the asthma series.

II = head of household living with wife who is included in the asthma series.

with water drains w.c. and central heating. Of these nine, five were heads of the household belonging to the asthma series eight of the nine were 55 years of age or older

### *Housing size*

Table 58 show the size of the apartments, according to the number of rooms, of the patients in the whole main asthma series. The table is compiled by the ranking method and distinguishes between heads of the household belonging to the asthma series and those belonging to the rest of the population, i.e. men who were

married to and lived with women belonging to the asthma series. The table does not give the number of occupants per apartment and thus gives no definite information with regard to overcrowding. More accurate information on the occurrence of overcrowding was obtained, however by another method, which will be described below. The table shows a relatively even distribution of the individuals according to the ranking scale with the reservation, however that in the two younger age groups and in the group of the oldest persons there is some predominance of those whose apartment size was less than that of the median control person.

Groups are by age 15	Groups acc. to standard of housing						
	1 (highest)	2	3	4	5	6	7 (lowest)
20-34	1 11	3	1			1	
35-39	1 11	1					
40-44	1 11	1					
45-49	1 11	2					
50-54	1 11	3	1				
55-59	1 11	10	1	1	1		2
60-64	1 11	2	1	2			
Total	1 11	13 22	2 3	2 2	1	2 1	

Table 57 Standard of housing 1960 according to classification 1-61 35 where the highest standard corresponds to group 1 and the lowest to group 7 for the married Uppsala urban district patient living with wife or husband respectively

1 = head of household, included in the asthma series.

11 = head of household, living with wife who is included in the asthma series.



ing to the calculation of sickness points, the mean "ranking figure" was 54.8 but here there appeared to be no noteworthy difference between the two groups described above.

Those patients belonging to groups A, B and C in Table 3 were studied in Table 56 where it was found that the mean figure for each of the groups exceeded 50.0. It is worthy of note that those heads of the household, belonging to the asthma series, in the groups B and C, i. e. the two groups of patients with a relatively high number of sickness points, had a mean ranking figure of a little below 50 while those belonging to group A had a remarkably high figure, i. e. 60. None of the groups shown in Table 56 differed significantly from the expected figure of 50 and no significant difference was shown between the pairs of subgroups.

The relationship between certain case history data and the housing standard at the time of the investigation was studied. Out of the 116 patients the 20 who had shown the highest ranking figures with regard to housing standard and the 20 who had shown the lowest were chosen. These two groups of patients were then studied for the purpose of finding out whether any of the eight case history factors mentioned on page 41 (sickness points, duration of disease etc.) had any statistically significant relationship with the housing standard of the patients.

The 20 patients who were recorded as having the highest housing standard had a mean ranking figure of 75.6 (range 68.0—84.3) while the 20 with

the lowest standard had a mean figure of 13.4 (range 4.0—21.8).

No statistically significant difference was shown between the two groups of patients for any of the factors studied. This meant therefore that with regard to the assessed degree of severity of the disease the 20 patients who were found to have the relatively lowest housing standard did not differ significantly as a group from the 20 with the relatively highest standard.

Table 57 shows the housing standard of the married Uppsala urban district patients, living with wife or husband. The classification into groups with different housing standards is based on the conditions shown in Table 54. The age of the women is given as that of their husbands. Table 57 allows comparison, within a group homogeneous from the point of view that it consists entirely of married urban dwellers, between the housing standard in the households where the head of the household belonged to the asthma series (the men) and that in the households where the head of the household belonged to the rest of the population (their wives belonging to the asthma series). The table shows that the asthma patients in all the age categories were well placed as a group, with a large majority in the highest standard group. None of them were placed in group 7. Of the heads of the household who belonged to the asthma series, 68.1 % lived in apartments belonging to standard group 1. The corresponding figure for the heads of the household living with their wives who belonged to the asthma series was 82.3 %. All except nine had an apartment

units living in one room unit. For the married persons in the asthma series, living with their wives or husbands, an expected number of 7.0 with overcrowded conditions was obtained, while the observed number was 3. This difference however was not significant. On calculating the expected value consideration was taken of the district in which each patient lived, also of civil state and age.

Of the three observed patients living in overcrowded conditions, one belonged to the urban population and the others lived in a rural district. Two of the three patients, one from the town and one from the country were heads of the household and belonged to the asthma series, while the head of the household but belonged to the rest of the population. The one was married to and living with a woman who was part of the asthma series. The expected number with overcrowded conditions among the asthma patients who were themselves head of the household was 2.8 and the observed number 2.0. The corresponding figures for those patients whose husbands acted as head of the household were 4.2 and 1.0 respectively. When the unmarried persons were included in the calculations the number of expected persons with overcrowded conditions increased, while the observed number still remained 1.0 since none of those belonging to this category in the asthma series could be regarded as living in overcrowded conditions, as defined above.

The question of overcrowding among the asthma patients was also studied on the basis of the definition made by Roos (1949) of the "ideal minimal housing standard" i.e. that a maximum of 1.5 person unit living in one room unit. It was then found that 20.7 % of the patients in the asthma series lived under overcrowded conditions. For comparison it may be mentioned that Småra and Ber-

genstam (1961) found that the corresponding figure for patients with osteogenesis imperfecta was 26.0 %.

To summarize it may be said that for this group of patients both the standard and the size of the housing were good. When the series of patients was divided into groups according to the assessed degree of severity of the disease no notable difference was shown between these groups. Even the standard of those receiving invalid pensions was equivalent to that of the total population. If anything overcrowding occurred to a lesser extent among the patients in the asthma series than in the total population. There was some tendency for those patients whose husbands were head of the household to have on an average a somewhat higher standard than those who themselves held this position. When a number of factors found typically in the case histories of asthma patients — were studied, none of them were found to have any statistically significant importance with regard to the housing standard at the time of the investigation.

#### *Smoking and alcohol consumption*

*Tobacco smoking* The smoking habits of the 116 patients (42 men and 74 women) in the asthma series were studied. Control information was obtained from the City of Uppsala Health Survey of 1961 in which 369 men and 263 women were studied. The method was essentially the same in the two investigations. The expected number of daily smokers (cigarettes, cigars or

Also shown in the table is the mean number of room units in the different groups according to so called ranking figures. The mean number of room units for the whole main series was 2.6.

The mean "ranking figure" for the housing size in the whole series (see Table 56) was 47.3 for that where the heads of the household belonged to the asthma series 46.3 and for that where the heads of the household belonged to the rest of the population 48.3.

For the invalid pensioners included in the series the mean figure was 37.0 the corresponding figure for the non

invalid pensioners was 49.0. The difference was not statistically significant. Those patients who belonged to groups B and C combined (see Table 56) attained a mean figure of 42.5, while the corresponding figure for those belonging to group A was considerably higher i.e. 57.3. The difference between group A and the combined groups B and C is significant (\*). A significant difference was also found between group A and the separate groups B and C.

A study was made of so-called over crowding this being defined in accordance with the population census of 1900 i.e. as more than 2.0 person

Groups according to age yrs.	Groups according to so-called ranking figures					Mean ranking figure	
	0-20	21-40	41-60	61-80	81-100		
30-34	I II	2 8	2		1	30 42.3	35.3
35-39	I II	2 8	2		1	31.4 33.8	36.3
40-44	I II				1 1	51.5 53.4	60.2
45-49	I II	1 1	1	2	1 1	54.8 48.3	55.0
50-54	I II		2 6	2	1 2	51.0 44.8	47.3
55-59	I II	3 3	0 5	3 1	4 3	49.5 49	51.0
60-64	I II	3 3	1	3 8	1 2	53.4 44.9	44.5
Mean number room units.							
Total I + II	1.2	1.8	2.0	3.6	5.2		2.0

Table 58. Size of living quarters (% of room units) 1900 according to age for each of the individuals in the asthma series expressed in accordance with the so-called ranking method (page 31) where each individual in the asthma series is compared with the so-called median person within the same age sex and civil status group in the total population (urban and rural districts population).

I = head of household, included in the asthma series.

II = head of household, living with wife who is included in the asthma series.

units living in one room unit. For the married persons in the asthma series, living with their wives or husbands an expected number of 2.0 with overcrowded conditions was obtained, while the observed number was 3. This difference however was not significant. On calculating the expected value consideration was taken of the district in which each patient lived, also of civil state and age.

Of the three observed patients with overcrowded conditions, one belonged to the urban population and the third lived in a rural district. Of the three patients, one from the town and one from the country were head of the household and belonged to the asthma series, while one was head of the household but belonged to the rest of the population. A few were married and living

with one who was a part of the asthma series. The expected number with overcrowded conditions among the asthma patients who were themselves head of the household was 2.6, and the observed number 2.0. The corresponding figures for those patients whose husbands acted head of the household were 4.2 and 1.0 respectively. When the unmarried persons were also included in the calculations the number of expected persons with overcrowded conditions increased, while the observed number still remained 2.0. When none of those belonging to this category in the asthma series could be regarded as living in overcrowded conditions, the deficit above

mentioned (1961) found that the corresponding figure for patients with osteogenesis imperfecta was 20.9 %.

*To summarize* it may be said that for this group of patients both the standard and the size of the housing were good. When the series of patients was divided into groups according to the assessed degree of severity of the disease no notable difference was shown between these groups. Even the standard of those receiving invalid pensions was equivalent to that of the total population. If anything overcrowding occurred to a lesser extent among the patients in the asthma series than in the total population. There was some tendency for those patients whose husbands were head of the household to have on an average a somewhat higher standard than those who themselves held this position. When a number of factors — found typically in the case histories of asthma patients — were studied none of them were found to have any statistically significant importance with regard to the housing standard at the time of the investigation.

#### *Smoking and alcohol consumption*

*Tobacco smoking* The smoking habits of the 116 patients (42 men and 74 women) in the asthma series were studied. Control information was obtained from the City of Uppsala Health Survey of 1961 in which 389 men and 333 women were studied. The method was essentially the same in the two investigations. The expected number of daily smokers (cigarettes, cigars or

The question of overcrowding among the asthma patients was also studied on the basis of the definition made by Roos (1949) of the ideal minimal housing standard, i.e. that with a maximum of 1.5 person unit living in one room unit. It was then found that 20.7 % of the patients in the asthma series lived under overcrowded conditions. For comparison it may be mentioned that Småra and Ber

pipe) of daily cigarette smokers among this group and also of non smokers were calculated. On calculating these expected figures consideration was taken of sex and age (age group range 5 years)

As seen in Table 59 the number of non smokers was highly significantly greater among the men in the asthma series than among the men in the control material. No noteworthy difference was seen in this respect with regard to the women.

Of the men and the women in the asthma series who were daily cigarette smokers, there were none who smoked more than 14 cigarettes per day. Of the daily cigarette smokers in the control material a few more than 20 % of the men and a few less than 5 % of the women smoked more than 14 cigarettes per day.

**Alcohol consumption** The alcohol consumption in the 116 patients in the asthma series was studied. As in the study of tobacco smoking the control material consisted of the persons who took part in the City of Uppsala Health Survey. In both the asthma series and the control material the groups of "total abstainers" and "almost total ab-

stainers" were selected. By "almost total abstainers" is meant persons who consume alcoholic drinks including beer less frequently than once a month. The observed number of total and almost total abstainers among the men was 18 while the expected number was 16. The corresponding figures for the women were 57 and 51 respectively. In calculating these figures consideration was taken of sex and age groups (group range 5 years).

To summarize the number of tobacco smokers among the men in the asthma series was highly significantly lower than among the men in the control material from the urban district of Uppsala. No noteworthy significance was found in this respect with regard to the women. None of the cigarette smokers smoked more than 14 cigarettes per day.

In the study of the alcohol consumption no significant difference was shown for the number of total abstainers and almost total abstainers between the asthma series and the control material.

	MALE		FEMALE	
	Observed N	Predicted N	Observed No	Predicted No
Non smokers	33	14	30	38
Daily smokers	10	28	15	10
of whom daily cigarette smokers	6	17	15	1

Table 59. Observed and predicted number of tobacco-smokers in the asthma series distributed according to sex.

The significance of difference from the predicted value is stated with significance asterisks.

## Discussion

The different parts of the investigation showed that there was surprisingly good social adaptation in this group of patients in spite of the often prolonged and severe disease. Certain factors however should not be forgotten. Perhaps the most important is the large average number of days of incapacity and symptom days reported by these patients. It is obviously impossible to state exactly what this meant in terms of economic burden and reduced production. Some information could certainly be obtained in most cases from the General Sickness Insurance or other sickness benefit societies. But the intermittent nature of the disease point to numerous short periods of illness, resulting in scattered absences from work. This must have affected income and production, and even placed a burden on the finances of the community. Calculations are unnecessary here and furthermore could not provide an accurate answer to these questions. On attempting to assess the economic significance of the disease consideration must be taken of all the journeys made for treatment, the often considerable cost of medicines, and doctors and hospital fees, which affect primarily the patient and secondarily the community. Especially badly affected are obviously those patients supported by invalid pensions. With respect to these persons in particular the burden on the community is also considerable.

It was not considered justified to make a separate description of the effects of the bronchial asthma on the respiratory and circulatory functions

in this group of invalid pensioners. The majority of them belonged to the group of patients who were assessed as having the highest degree of severity of the disease i.e. they had attained 2000 sickness points or more (group C). As expected these patients also had on an average a low ventilation capacity pronounced signs of hyperinflation and a low physical work capacity while the hemodynamic findings did not differ in any noteworthy degree from the rest of the material or from ordinary conditions. As regards the estimated degree of severity of the bronchial asthma, these patients did not on an average differ notably from the rest of the material.

This series of patients with bronchial asthma was studied from several different aspects. A series of this kind is unavoidably heterogeneous in certain respects. For example as mentioned previously there were large variations both in the ages of the patients and the ages at which the asthma was first manifested. There were also considerable variations with regard to other factors, for example the duration of the disease up to the time of the investigation. Because of this it was not always possible to use the material in its entirety for studies of the effect of the disease on the social situation of these patients. In the case of family formation, for example a serious disease occurring at a certain age period may have a determining influence. For several of the factors indicating social adaptation, therefore, studies were made on a part or parts of the material that were considered especially suit-

pipe) of daily cigarette smokers among this group and also of non smokers were calculated. On calculating these expected figures consideration was taken of sex and age (age group range 5 years)

As seen in Table 59 the number of non smokers was highly significantly greater among the men in the asthma series than among the men in the control material. No noteworthy difference was seen in this respect with regard to the women

Of the men and the women in the asthma series who were daily cigarette smokers, there were none who smoked more than 14 cigarettes per day. Of the daily cigarette smokers in the control material a few more than 20 % of the men and a few less than 5 % of the women smoked more than 14 cigarettes per day

**Alcohol consumption** The alcohol consumption in the 116 patients in the asthma series was studied. As in the study of tobacco smoking the control material consisted of the persons who took part in the City of Uppsala Health Survey. In both the asthma series and the control material the groups of total abstainers and "almost total ab-

stainers were selected. By almost total abstainers is meant persons who consume alcoholic drinks, including beer less frequently than once a month. The observed number of total and almost total abstainers among the men was 18, while the expected number was 16. The corresponding figures for the women were 57 and 57 respectively. In calculating these figures consideration was taken of sex and age groups (group range 5 years)

To summarize the number of tobacco smokers among the men in the asthma series was highly significantly lower than among the men in the control material from the urban district of Uppsala. No noteworthy significance was found in this respect with regard to the women. None of the cigarette smokers smoked more than 14 cigarettes per day

In the study of the alcohol consumption no significant difference was shown for the number of total abstainers and almost total abstainers between the asthma series and the control material

	MALE		FEMALE	
	Observed No	Predicted No	Observed No	Predicted No
Non smokers	32	14	50	38
Daily smokers	10 **	28	16	19
of whom daily cigarette smokers	8	17	16	17

Table 59 Observed and predicted number of tobacco-smokers in the asthma series distributed according to sex.

The significance of difference from the predicted value is stated with significance asterisks.

## Discussion

The different parts of the investigation showed that there was surprisingly good social adaptation in this group of patients in spite of the often prolonged and severe disease. Certain factors however should not be forgotten. Perhaps the most important is the large average number of days of incapacity and symptom days reported by these patients. It is obviously impossible to state exactly what this meant in terms of economic burden and reduced production. Some information could certainly be obtained in most cases from the General Sickness Insurance or other sickness benefit societies. But the intermittent nature of the disease points to numerous short periods of illness, resulting in scattered absences from work. This must have affected income and production, and even placed a burden on the finances of the community. Calculations are unnecessary here and furthermore could not provide an accurate answer to these questions. On attempting to assess the economic significance of the disease consideration must be taken of all the journeys made for treatment, the often considerable cost of medicines, and doctors' and hospital fees, which affect primarily the patient and secondarily the community. Especially badly affected are obviously those patients supported by invalid pensions. With respect to these persons in particular the burden on the community is also considerable.

It was not considered justified to make a separate description of the effects of the bronchial asthma on the respiratory functions

in the group of invalid pensioners. The majority of them belonged to the group of patients who were assessed as having the highest degree of severity of the disease i.e. they had attained 2000 sickness points or more (group C). As expected, these patients also had on an average a low ventilation capacity, pronounced signs of hyperinflation and a low physical work capacity while the hemodynamic findings did not differ to any noteworthy degree from the rest of the material or from ordinary conditions. As regards the estimated degree of severity of the bronchial asthma, these patients did not on an average differ notably from the rest of the material.

This series of patients with bronchial asthma was studied from several different aspects. A series of this kind is unavoidably heterogeneous in certain respects. For example as mentioned previously there were large variations both in the ages of the patient and the ages at which the asthma was first manifested. There were also considerable variations with regard to other factors, for example the duration of the disease up to the time of the investigation. Because of this it was not always possible to use the material in its entirety for studies of the effect of the disease on the social situation of these patients. In the case of family formation, for example, a serious disease occurring at a certain age period may have a determining influence on several of the factors indicating social adaptation. Therefore studies were made in a part or parts of the material that were considered especially suit-



able. Throughout the whole investigation, however, the main interest was concentrated on the question of the extent to which bronchial asthma had influenced the possibilities of these persons to adapt themselves socially.

A small number of the patients had had relatively few symptom days and days of incapacity and from a social adaptation point of view these patients were obviously not as interesting as those who for several years had had frequent more or less prolonged periods of disablement. They were not excluded from the material however since it was considered best not to make an exception from the rule that hospital admission during the given period permitted participation in the investigation. Even if the course of their disease was not severe, they had previously had asthma symptoms of such a degree that admission to hospital was considered necessary. It seems probable that the experience of asthmatic attacks and the knowledge that they suffered from a usually prolonged disease might have had some inhibitory influence on the development of these persons with a result that their social situation could be affected. It seemed of interest to find out whether there were any patients whose condition had improved spontaneously or whether the asthma had possibly changed in character so that the need for urgent hospital care was reduced or no longer existed.

Ask Upmark (1957) states that the annual number of deaths from bronchial asthma in Great Britain is estimated at 7 per 100 000 inhabitants. The corresponding figure for the U.S.A. is 1.6

Ask Upmark considers it probable that the mortality in Sweden is not as high as that in the U.S.A., but that the disease gives rise to a great degree of human suffering and that by its recurrent nature and long periods of symptoms it constitutes a handicap to production and national economy. Some information on the mortality in asthma and related disorders has also been given by Williams (1953) Groen and Lieber (1960) Bower (1961) York (1962) and Walkup and Connolly (1963). A number of authors have published series of fatal cases. Huber and Koessler (1922) were among the first of them. Since this time several workers have discussed deaths from asthma and reviewed the literature, e.g. Unger (1952) Earle (1953) Alexander (1958) Williams and Leopold (1959) and Cardell and Pearson (1959).

The results obtained from earlier published studies on the mortality in asthma contribute only to a small extent to the elucidation of the problems of the present investigation. No investigation appears to have been made on the mortality in bronchial asthma alone, where the observations were made during a certain period of time and where the observed mortality was compared with the expected figure, taking into consideration age and sex. 15 of the 176 patients of the present asthma series died, compared with an expected figure of 10.3. If the five cases in whom an actual asthmatic attack was considered to be the direct cause of death are excluded there remain ten deaths, which is almost equal to the expected number. This in

dicates — if the cases in whom the asthma in itself was considered to be the direct cause of death are disregarded — that the disease did not have had the indirect effect of increasing the mortality.

No definite reason for the relatively small tendency of the asthma patients to move to other districts can be given, but there were probably several contributory factors. It is reasonable to assume that patients with this disease avoid taking certain risks. Moving from one place to another is often connected with a change of employment. For a person with a chronic disease there is probably often little attraction in the idea of taking the risks that may be associated with moving, when this means leaving a milieu and place of employment that he has perhaps been accustomed to for a long period.

For many patients moving would also mean losing contact with the doctor upon whom he might have relied for several years. The knowledge that his own doctor and possibly the hospital that his essential data, would probably be available in an emergency is certainly of great importance to many asthma patients.

On the other hand it would not have been surprising to find that these patients had moved to a greater extent than the rest of the population. The change of climate which since of old has often been recommended to persons suffering from asthma is a factor to be considered, but this does not appear to have been of any importance in this series of patients. The reason may possibly be — which was often

evident from discussions with the patients — that for many patients occasional visits to other places did not prove to have the desired effect on their asthma.

Another reason for expecting that these patients might have a greater tendency to move to other places is that this disease might be expected to predispose the patient to a change of occupation. This factor appeared to be of little importance in this series, however possibly due to the fact, as mentioned previously that moving to another district often entails certain risks, and also to the fact that the labour market was favourable and other work was therefore available at closer quarters.

It is natural for older persons to feel so settled in their milieu that they do not often entertain the idea of moving. The results of the present study showed, however that it was not only among the older patients that there was a high frequency of those who continued to live in Uppsala. Thus it was not predominance of the older age groups that gave the relatively high frequency of those who continued to live in Uppsala, in the asthma series as a whole.

Part of the material was studied with regard to *general education*. For this study only those patients who had symptoms of asthma at the ages of 16–24 years (see page 122) were included. Here, as in the study of the effect of the asthma on the formation of families, the material was small, but it was nevertheless decided to study this factor in order to shed as much

light as possible on the significance of the disease from the social adaptation point of view

No significant difference was found with regard to the number of lower certificate and matriculation examinations passed between the asthma series and the total population. There was a possibly tendency to a somewhat greater number of examinations passed among the asthma patients. There appears therefore to be some support for the assumption that these patients who had symptoms of asthma at the ages 16—24 years regarded as a group had just as good a general education as the total population. The reason that the patients had, on an average such a good education could of course have been that they had special intellectual or social prerequisites. There is, however no support for such an assumption. The reason may also possibly be that these patients or their parents, in their effort to avoid the type of work in which there was greater demand on physical strain chose training of a more intellectual nature. On the other hand there were probably some pupils who because of the severity of the disease had difficulty in attending the school lessons to a sufficient extent, and were therefore more badly situated in the competition for marks and examinations. In the individual asthma patients it was seen that their development was influenced sometimes more by one and sometimes more by another of these factors, and for the group as a whole the factors causing difficulties appeared to be more than well compensated.

It was mentioned on giving the

results of this study that the favourable conditions for education in Uppsala may have influenced the number of lower certificate and matriculation examinations passed by patients in this series. The significance of this good access to education was reduced somewhat for this material however by the fact that some of the patients studied did not move to Uppsala until after the age of 18 years. In the investigation made on the number of patients who had a university education or its equivalent however the control material consisted of the total population of Uppsala, and a direct comparison between the asthma series and the control material was not therefore affected by the advantages of the local educational conditions.

The number of patients in the asthma series who were or had been married agreed well with the expected figure which was calculated with regard to sex and age and also to district i. e. whether the patient belonged to the urban or rural population — this because the marriage frequency particularly for the men, was lower in the rural districts than in the town. Since in a large number of the patients in the asthma series the onset of the disease occurred after the age of 30 years — the age when the majority of all marriages have already taken place — it was considered more suitable in studying the effect of the asthma on the marriage frequency to select a material of patients in whom the asthma was first manifested at or before the age of 21 years and who during the ages 20—29 years had no more than two symptom free years. In this group

also there was very good agreement between the observed and the expected numbers of those who had at any time been married. It had appeared possible here that for these patients the asthma might have constituted a burden both with regard to meeting members of the opposite sex and in the question of liabilities for maintenance. The fact that these assumptions were not supported by the results may be connected with the usually intermittent course of the disease which may include periods when the symptoms are absent or only mild.

This factor might also be expected to be of importance with regard to the attitude of the patients towards the greater maintenance burden that accompanies the addition of children to the family. The group studied here consisted of married patients, living with their husbands or wives — in age groups where it may be assumed that the overwhelming majority of the children are below the age of 16 years. In these selected patients the asthma was first manifested at or before the age of 21 years, and during the ages 20—29 years they had had at the most two symptom-free years. No difference was shown with regard to the number of children under the age of 16 years in a comparison with the corresponding age groups in the total populations of the urban and rural districts respectively. It would have been understandable if this figure had been lower in the asthma group when considering that for the men the responsibility of supporting a family might have constituted an inhibitory factor to having children, and that for the women it is

conceivable that fear of manifestations of the disease might have caused some fear of pregnancy and delivery.

The tendency to fewer divorced persons in the asthma series than in the total population was relatively marked. The difference between the observed and expected numbers of those who had at any time been divorced was statistically significant. Bruce Dahlström and Uggla (1960) showed in a study of tuberculous patients that a remarkably large number were single or divorced. Of the men of ages 45—85 years no fewer than 25 % belonged to the former and just as many to the latter category. The corresponding figures for the total population were given as 10 % single persons and 7 % divorced.

There are however some striking differences between the two series which must first be emphasized. The frequently large consumption of alcohol among the tuberculous patients, the long periods of hospital care often needed by patients with the disease, and the biased ideas still associated with this diagnosis are some examples indicating that direct comparison with asthma patients is not justifiable in all respects.

It might thus seem surprising that the number of patients in the asthma series living as divorced was fewer than the expected number. It is only possible to present a few suppositions as an explanation. Bronchial asthma is a prolonged and intermittent disease, that usually requires lots of consultations and repeated short periods of hospital care, and it is possible that in reality this may lead to a high degree

light as possible on the significance of the disease from the social adaptation point of view

No significant difference was found with regard to the number of lower certificate and matriculation examinations passed between the asthma series and the total population. There was a possibly tendency to a somewhat greater number of examinations passed among the asthma patients. There appears therefore to be some support for the assumption that these patients who had symptoms of asthma at the ages 16—24 years, regarded as a group had just as good a general education as the total population. The reason that the patients had, on an average, such a good education could of course have been that they had special intellectual or social prerequisites. There is, however, no support for such an assumption. The reason may also possibly be that these patients or their parents in their effort to avoid the type of work in which there was greater demand on physical strain chose training of a more intellectual nature. On the other hand there were *probably some pupils who because of the severity of the disease had difficulty in attending the school lessons to a sufficient extent, and were therefore more badly situated in the competition for marks and examinations.* In the individual asthma patients it was seen that their development was influenced sometimes more by one and sometimes more by another of these factors and for the group as a whole the factors causing difficulties appeared to be more than well compensated.

It was mentioned on giving the

results of this study that the favourable conditions for education in Uppsala may have influenced the number of lower certificate and matriculation examinations passed by patients in this series. The significance of this good access to education was reduced somewhat for this material, however, by the fact that some of the patients studied did not move to Uppsala until after the age of 18 years. In the investigation made on the number of patients who had a university education or its equivalent however the control material consisted of the total population of Uppsala, and a direct comparison between the asthma series and the control material was not therefore affected by the advantages of the local educational conditions.

The number of patients in the asthma series who were or had been married agreed well with the expected figure which was calculated with regard to sex and age and also to district i.e. whether the patient belonged to the urban or rural population — this because the marriage frequency particularly for the men was lower in the rural districts than in the town. Since in a large number of the patients in the asthma series the onset of the disease occurred after the age of 30 years — the age when the majority of all marriages have already taken place — it was considered more suitable in studying the effect of the asthma on the marriage frequency to select a material of patients in whom the asthma was first manifested at or before the age of 21 years and who during the ages 20—29 years had no more than two symptom free years. In this group

also there was very good agreement between the observed and the expected numbers of those who had at any time been married. It had appeared possible here that for these patients the asthma might have constituted a burden both with regard to meeting members of the opposite sex and in the question of liabilities for maintenance. The fact that these assumptions were not supported by the results may be connected with the usually intermittent course of the disease which may include periods when the symptoms are absent or only mild.

This factor might also be expected to be of importance with regard to the attitude of the patients towards the greater maintenance burden that accompanies the addition of children to the family. The group studied here consisted of married patients, living with their husbands or wives — in age groups where it may be assumed that the overwhelming majority of the children are below the age of 16 years. In these selected patients the asthma was first manifested at or before the age of 21 years, and during the ages 20—29 years they had had at the most two symptom free years. No difference was shown with regard to the number of children under the age of 16 years in a comparison with the corresponding age groups in the total populations of the urban and rural districts respectively. It would have been understandable if this figure had been lower in the asthma group when considering that for the men the responsibility of supporting a family might have constituted an inhibitory factor to having children, and that for the women it is

conceivable that fear of manifestations of the disease might have caused some fear of pregnancy and delivery.

The tendency to fewer divorced persons in the asthma series than in the total population was relatively marked the difference between the observed and expected numbers of those who had at any time been divorced was statistically significant. Bruce Dahlström and Uggla (1960) showed in a study of tuberculous patients that a remarkably large number were single or divorced. Of the men of ages 45—65 years no fewer than 25 % belonged to the former and just as many to the latter category. The corresponding figures for the total population were given as 10 % single persons and 7 % divorced.

There are, however, some striking differences between the two series which must first be emphasized. The frequently large consumption of alcohol among the tuberculous patients, the long periods of hospital care often needed by patients with the disease and the biased ideas still associated with this diagnosis are some examples indicating that direct comparison with asthma patients is not justifiable in all respects.

It might thus seem surprising that the number of patients in the asthma series living as divorced was fewer than the expected number. It is only possible to present a few suppositions as an explanation. Bronchial asthma is a prolonged and intermittent disease that usually requires lots of consultations and repeated short periods of hospital care, and it is possible that in reality this may lead to a high degree

light as possible on the significance of the disease from the social adaptation point of view.

No significant difference was found with regard to the number of lower certificate and matriculation examinations passed between the asthma series and the total population. There was a possibly tendency to a somewhat greater number of examinations passed among the asthma patients. There appears therefore to be some support for the assumption that these patients who had symptoms of asthma at the ages 16—24 years regarded as a group had just as good a general education as the total population. The reason that the patients had on an average such a good education could of course have been that they had special intellectual or social prerequisites. There is, however, no support for such an assumption. The reason may also possibly be that these patients or their parents, in their effort to avoid the type of work in which there was greater demand on physical strain chose training of a more intellectual nature. On the other hand there were probably some pupils who because of the severity of the disease had difficulty in attending the school lessons to a sufficient extent, and were therefore more badly situated in the competition for marks and examinations. In the individual asthma patients it was seen that their development was influenced sometimes more by one and sometimes more by another of these factors, and for the group as a whole the factors causing difficulties appeared to be more than well compensated.

It was mentioned on giving the

results of this study that the favourable conditions for education in Uppsala may have influenced the number of lower certificate and matriculation examinations passed by patients in this series. The significance of this good access to education was reduced somewhat for this material, however, by the fact that some of the patients studied did not move to Uppsala until after the age of 18 years. In the investigation made on the number of patients who had a university education or its equivalent, however, the control material consisted of the total population of Uppsala and a direct comparison between the asthma series and the control material was not therefore affected by the advantages of the local educational conditions.

The number of patients in the asthma series who were or had been married agreed well with the expected figure, which was calculated with regard to sex and age and also to district, i. e. whether the patient belonged to the urban or rural population — this because the marriage frequency particularly for the men, was lower in the rural districts than in the town. Since in a large number of the patients in the asthma series the onset of the disease occurred after the age of 30 years — the age when the majority of all marriages have already taken place — it was considered more suitable in studying the effect of the asthma on the marriage frequency to select a material of patients in whom the asthma was first manifested at or before the age of 21 years and who during the ages 20—29 years had no more than two symptom free years. In this group

to stop working or his employer asked him to leave at least for a time. When later the asthma reached a calmer stage there was not always need for the patient at his old place of employment and he had to obtain a new job, but there was often an opportunity here of choosing work of a type more suitable with regard to the disease. This situation probably applied to a greater extent to the less qualified persons than to the more highly qualified. Other patients changed their occupations for preventive reasons — often to avoid certain factors that had previously acted provokingly on the latent asthma.

The frequency of *economically active married women* was on the whole the same in the asthma series as in the corresponding population. It must be regarded as remarkable that this group of women including many who for several years had been completely or partly disabled by asthma, were now to be found to such a great extent among those who were carrying out income yielding work. In many of them the disease had improved during the latter years and this had provided an opportunity of breaking the isolation that had previously been unavoidable on account of repeated frequent periods of asthma symptoms. This probably explains, at least partly the fact that such a relatively large number of these women were found to be working. It was gratifying to find that this form of readaptation was possible for several women in spite of the fact that often only one or a few years had elapsed since a time when the asthma was so severe that income-

yielding work was almost out of the question.

It may be assumed that the distribution into sickness benefit classes by the General Sickness Insurance gives a good idea of the differences in the mean income levels of groups of individuals. The information given by the members to the General Sickness Insurance with regard to their income is checked to a great extent with that given to the income tax authorities.

On studying the income level in the group of economically active men and women in 1963, it was found that the mean income level in December of that year was for the men practically the same as that of the men in the control material. The women in the asthma series, on the other hand, had on an average a somewhat lower income level than expected.

It was found that eight years earlier i. In December 1945 the income level of the men in the asthma series, born in 1897 or later who in 1963 were not receiving an invalid pension and were not self-employed, seemed to be lower than that of the corresponding median person in the total population by an average of about 20 %, consideration being taken of age, sex and district of residence. In December 1960 this group of patients had caught up to such an extent that the difference was reduced by about half and after a further three years the income level of the median person in the control material had been reached on an average by these patients in the asthma series. Even if this tendency to an improvement in income did not reach the significance level it appeared to correspond well with the



of solidarity and unity between the husband and wife. It is certainly possible that the situation of the patient with attack after attack of severe asthma could produce a strong feeling of sympathy in the husband or wife. The patient, on his side, is obviously in a situation that is often characterized by need of help whether this be assistance during acute attacks or long term help e.g. maintenance etc.

The distribution between the different branches of industry among the economically active patients in the asthma series offered no indication that these individuals differed from the rest of the population in this respect. The distribution of the economically active patients between the two occupational positions of employer and employee showed an over representation in the former group for the asthma series when compared with the total population. One possible contributory explanation is that the employer with his more independent position is able to plan his work with regard to his disease and that handicapped persons often therefore choose this form of position. The fact that the number of persons receiving invalid pensions was slightly greater among the group of employees in this material also supports the view that the employer may have greater possibilities of reconciling the disease with his work. A tendency to over representation in the group of employers was also found in the study by Smårs and Berfenslarn (1961) of patients with osteogenesis imperfecta and it is highly probable that this effort to obtain work of an independent

nature is characteristic of handicapped persons

The social consequences of asthma are seen perhaps especially clearly by the fact that approximately every fourth man in the present series was forced to *discontinue income-yielding work* permanently on account of asthma and furthermore that approximately every third man had to *change his occupation* for the same reason. A notable number of the men had left agricultural and forestry work, either when they permanently stopped working or on changing their occupations. Since the actual distribution of the economically active men in the asthma series appeared to indicate some degree of over representation in these branches of industry this means that a large number of the men earned or had previously earned their living from agriculture and forestry work. This may seem reasonable — from a causative aspect — in consideration of the special position of this type of work with regard to allergies.

Several of the women in the material were also affected by the disease in a similar way but not however to the same extent numerically as the men since the women did not have steady permanent work to the same extent as the men. Further the social consequences in the women were probably of less significance than in the men, since the latter are more often responsible for supporting the family.

The reason that asthma necessitated changes in occupation was in some cases that the disease had reduced the working capacity to such an extent that either the patient himself decided

unity of studying the effect of the patient's asthma on her family. It is conceivable that this latter effect may be expressed in the fact that a relatively large proportion of the economic resources of the family is allotted to the home. If this was in fact so it would support the view that the asthma influenced these patients or their families to improve their housing standard.

It may also be asked whether special financial benefits from the community which may be received by some patients, e. g. invalid pensioners, could have contributed to the good average housing standard. A further contributory factor may be that the disease demanded a relatively large degree of assiduity. This could perhaps have contributed partly to the fact that the patient had been able to retain an apartment of good class, or to obtain a different one of a better standard than the old. It may however be mentioned that for the invalid pensioners the average difference from the housing also for the median person in the total population almost reached the limit of significance.

In presenting the results of the majority of the studies on different indicators of social adaptation, those 12 patients who had other chronic diseases — in addition to the asthma — which could have had some determining influence on his life. In general, his health condition and functions were excluded. This group of patients did not differ appreciably from the rest of the material however with regard to the indicators studied.

It is obvious that to a group of patients such as those in the asthma series the possibilities of good medical care and general support in cases of employment difficulties or disablement, offered by the community must have been of significance with regard to their social situation. The fact that the two decades preceding the time of the present investigation were on the whole characterized by a favourable labour market contributed to the relatively good opportunities of the handicapped to assert themselves in the competition for employment. This factor applied especially to the economically active persons, and less to those receiving invalid pensions. It was obvious that in the case of many of the latter persons the support of the community, the family relatives and others was of decisive importance.

Whether this series of patients had on an average better intellectual prerequisites than the total population was not clear. Certain factors appear to indicate that the disease resulted in some concentration on education and professional training with an aim at more favourable positions, and it is possible that this could also be connected with the fact that the disease demanded an assiduous manner of living. No psychiatric investigations were made on this material but on many occasions of personal contact and often detailed conversations with the patients mainly during the collection of the material, they gave on the whole the impression of good mental balance.

The results obtained from the studies of the different indicators used to give

improvement as regards the asthma noted for the majority of the patients on taking their case histories, consideration being taken of the estimated number of days of incapacity due to asthma and days with asthma symptoms during earlier periods.

Increased prosperity in a community is to a great part expressed by a raised *standard of housing* which may probably be said to constitute an important measure of the social situation of a person or group of persons. This is not only because in the majority of cases the housing standard of a person is related to his income and economic situation, but also because generally speaking this standard constitutes some indication of his ambition to organize a satisfactory milieu for himself and his family. Even when the asthma material was divided into sub-series so that the mean housing standard for certain groups that were especially badly affected by the asthma e.g. invalid pensioners, was studied separately it was shown that these groups compared relatively well with the total population, even if there was often some difference between those patients in the series who were themselves heads of the household and those whose husbands held this position. The latter category constantly showed a somewhat higher mean housing standard than the corresponding total population which appeared to indicate that those families in which the wife had asthma made a special endeavour to keep up a good standard. It seems to be rather more remarkable that the group of invalid pensioners on an average did not have a lower housing

standard than the total population. When, however a study was made of those persons who at the time of the housing census (1960) had the highest mean number of sickness points per year (1957—61) it was found that this group on an average showed the lowest housing standards of the asthma series. Not for these patients either however was the average difference from the housing standard reached by the median person in the total population particularly large. When the influence of a number of factors — found typically in the case history of asthma patients — were studied none of them were found to have any statistically significant importance with regard to the housing standard at the time of the investigation.

It may be asked whether the frequently discussed relationship between asthma and the housing standard (damp draughts etc.) may constitute some reason for the good housing situation found on an average among these patients. In other words do asthma patients often make a special effort to obtain good housing conditions in the hope that this will have a favourable effect on the course of the disease? Conversations with the patients in this series indicated that this was true for several of them. Some support for this supposition appears to be offered by the fact mentioned above that there was some tendency — not however significant — to a relatively high housing standard in the group of married women of the series, living with their husbands. The study of this group is of especial interest in this respect since to some extent it provides an opport

ality of studying the effect of the patient's asthma on her family. It is conceivable that this latter effect may be expressed in the fact that a relatively large proportion of the economic resources of the family is allotted to the home. If this was in fact so, it would support the view that the asthma influenced these patients or their families to improve their housing standard.

It may also be asked whether special financial benefits from the community which may be received by some patients, e. g. in old pensioners, could have contributed to the good average housing standard. A further contributory factor may be that the disease demanded a relatively large degree of assiduity. This could perhaps have contributed partly to the fact that the patient had been able to retain an apartment of good class, or to obtain a different one of a better standard than the old. It may however be mentioned that for the invalid pensioners the average difference from the housing size for the median person in the total population almost reached the limit of significance.

In presenting the results of the majority of the studies on different indications of social adaptation, those 12 patients who had other chronic diseases — in addition to the asthma — which could have had some determining influence on his life in general, his health condition and functions were excluded. This group of patients did not differ appreciably from the rest of the material, however with regard to the indicators studied.

It is obvious that to a group of patients such as those in the asthma series the possibilities of good medical care and general support in cases of employment difficulties or disablement, offered by the community must have been of significance with regard to their social situation. The fact that the two decades preceding the time of the present investigation were on the whole characterized by a favourable labour market contributed to the relative good opportunities of the handicapped to assert themselves in the competition for employment. This factor applied especially to the economically active persons, and less to those receiving invalid pensions. It was obvious that in the case of many of the latter persons the support of the community, the family relatives and others was of decisive importance.

Whether this series of patients had on an average better intellectual prerequisites than the total population was not clear. Certain factors appear to indicate that the disease resulted in some concentration on education and professional training with an aim at more favourable positions, and it is possible that this could also be connected with the fact that the disease demanded an assiduous manner of living. No psychiatric investigations were made on this material, but on the many occasions of personal contact and often detailed conversations with the patients mainly during the collection of the material, they gave on the whole the impression of good mental balance.

The results obtained from the studies of the different indicators used to give

Improvement as regards the asthma noted for the majority of the patients on taking their case histories, consideration being taken of the estimated number of days of incapacity due to asthma and days with asthma symptoms during earlier periods

Increased prosperity in a community is to a great part expressed by a raised *standard of housing* which may probably be said to constitute an important measure of the social situation of a person or group of persons. This is not only because in the majority of cases the housing standard of a person is related to his income and economic situation but also because generally speaking this standard constitutes some indication of his ambition to organize a satisfactory milieu for himself and his family. Even when the asthma material was divided into sub-series so that the mean housing standard for certain groups that were especially badly affected by the asthma e.g. invalid pensioners, was studied separately it was shown that these groups compared relatively well with the total population, even if there was often some difference between those patients in the series who were themselves heads of the household and those whose husbands held this position. The latter category constantly showed a somewhat higher mean housing standard than the corresponding total population which appeared to indicate that those families in which the wife had asthma made a special endeavour to keep up a good standard. It seems to be rather more remarkable that the group of invalid pensioners on an average did not have a lower housing

standard than the total population. When however a study was made of those persons who at the time of the housing census (1960) had the highest mean number of sickness points per year (1957-61) it was found that this group on an average showed the lowest housing standards of the asthma series. Not for these patients either however was the average difference from the housing standard reached by the median person in the total population particularly large. When the influence of a number of factors — found typically in the case history of asthma patients — were studied, none of them were found to have any statistically significant importance with regard to the housing standard at the time of the investigation.

It may be asked whether the frequently discussed relationship between asthma and the housing standard (damp, draughts etc.) may constitute some reason for the good housing situation found on an average among these patients. In other words do asthma patients often make a special effort to obtain good housing conditions in the hope that this will have a favourable effect on the course of the disease? Conversations with the patients in this series indicated that this was true for several of them. Some support for this supposition appears to be offered by the fact, mentioned above, that there was some tendency — not, however significant — to a relatively high housing standard in the group of married women of the series, living with their husbands. The study of this group is of especial interest in this respect, since to some extent it provides an opport

quality of studying the effect of the patient's asthma on her family. It is conceivable that this latter effect may be expressed in the fact that a relatively large proportion of the economic resources of the family is allotted to the home. If this was in fact so, it would support the view that the asthma influenced these patients or their families to improve their housing standard.

It may also be asked whether special financial benefits from the community which may be received by some patients, e.g. invalid pensioners, could have contributed to the good average housing standard. A further contributory factor may be that the disease demanded a relatively large degree of assiduity. This could perhaps have contributed partly to the fact that the patient had been able to retain an apartment of good class, or to obtain a different one of a better standard than the old. It may however be mentioned that for the invalid pensioners the average difference from the housing size for the median person in the total population almost reached the limit of significance.

In presenting the results of the majority of the studies on different indications of social adaptation, those 12 patients who had other chronic diseases — in addition to the asthma — which could have had some determining influence on his life in general, his health condition and functions were excluded. This group of patients did not differ appreciably from the rest of the material, however with regard to the indicators studied.

It is obvious that to a group of patients such as those in the asthma series the possibilities of good medical care and general support in cases of employment difficulties or disablement offered by the community must have been of significance with regard to their social situation. The fact that the two decades preceding the time of the present investigation were on the whole characterized by a favourable labour market contributed to the relatively good opportunities of the handicapped to assert themselves in the competition for employment. This factor applied especially to the economically active persons and less to those receiving invalid pensions. It was obvious that in the case of many of the latter persons the support of the community, the family relatives and others was of decisive importance.

Whether this series of patients had on an average better intellectual prerequisites than the total population was not clear. Certain factors appear to indicate that the disease resulted in some concentration on education and professional training with an aim at more favourable positions, and it is possible that this could also be connected with the fact that the disease demanded an assiduous manner of living. No psychiatric investigations were made on this material but on the many occasions of personal contact and often detailed conversations with the patients mainly during the collection of the material, they gave on the whole the impression of good mental balance.

The results obtained from the studies of the different indicators used to give

an idea of the adaptation to the community of this group of patients, provide a perhaps unexpectedly bright picture. It must be remembered, however, on reviewing the effects of this disease, that a certain degree of excess mortality — but not significant — has been

shown. It must also be remembered that from a social point of view the effects of this disease were felt to a great extent by many patients. No fewer than every fifth patient had received an invalid pension on account of asthma.

### Summary

No systematic socio-medical investigations on the effects of bronchial asthma on the adaptation of the patient to the community appear to have been made previously. A report is made here of a socio-medical study of persons who were still living in Uppsala and its surrounding rural districts after a certain period of time and who had been previously admitted to an Uppsala hospital for the treatment of bronchial asthma. In some parts of the investigation, namely the study of the frequency of changes of residence and the mortality study, those who had moved to other districts were also included. 128 patients were interviewed and examined personally by the author. 12 of them had or had had other chronic bronchial diseases, apart from the bronchial asthma, and these patients were therefore not included in the general analyses of the results of studies of different indicators of social adaptation.

Some degree of excess mortality was shown, but this was not significant. Of the 15 patients in this series who died (expected number 10.3) it was considered that 5 had died as a direct result of asthmatic attacks. If those cases are disregarded, in which the bronchial

asthma in itself was considered to be the direct cause of death, the disease thus did not appear to have had the indirect effect of increasing the mortality rate. Approximately every fifth patient was receiving an invalid pension solely or mainly by reason of asthma. These patients constituted a group in which both the patient himself and the community were considerably burdened by the social consequences of the disease.

For the purpose of finding out the extent to which these patients moved to other districts, a study was made on those who, on admission to hospital, were living in the Uppsala urban district. The factors that might be assumed to influence the patients to remain in their own district or move to another

The standard  
still live

weight  
ing is  
signif  
les th  
contro  
person  
at r  
ule.

T  
in  
ents,

was manifested at or before the age of 16 years, appeared satisfactory when assessed by the number of lower school certificate and matriculation examinations passed. No noteworthy difference was shown between the asthma series and the total population as regards the number of persons with a university education. This comparison was based on information from official statistics, including the population census of 1960. Some factors that might be expected to inhibit or promote education in a group of patients such as this were discussed.

The marriage frequency was of the same order of size as that in the total population. This also held for the small number of patients in whom the asthma was manifested at or before the age of 21 years and who suffered from symptoms of this disease during the ages 21–30 years. In a study of the men and women in this latter group, who were born in 1921 or later and 1924 or later respectively — i.e. the age groups in which the overwhelming majority of children were below 16 years of age — it was found that the number of children below that age did not differ significantly from the expected number. The patients in the asthma series showed a relatively marked tendency to fewer divorces than the total population. For the studies of the marriage frequency, number of children and number of divorces, information for the total population was obtained from the 1960 population census. For the number of divorces, information was also obtained from the registrar's office.

The distribution between the differ

ent branches of industry among the economically active patients of the asthma series gave no indication that these patients differed from the total population in this respect. A study of the distribution between the two occupational positions of employer and employee showed an over-representation in the group of employers among the patients in the asthma series when compared with the total population. In these two studies also, information was obtained from the 1960 population census. Over half of the men found it necessary either to *discontinue income yielding work or change their occupation* on account of asthma, a fact which in itself strongly emphasizes the way in which this disease affected many patients. A report is made of the changes of occupation among these patients, and the reasons are discussed. There was an obvious tendency for the patients to change over to work involving less physical strain.

The frequency of married women with income-yielding work agreed in the main with that in the corresponding total population, in spite of the fact that the working capacity of many of the women in the asthma series had often been greatly reduced for several years.

The income level of the economically active patients in the asthma series was studied with the aid of the classification made by the General Sickness Insurance into sickness benefit groups. No noteworthy difference was found between the men and the control material. The women, however, appeared to have a lower income level. A study was made of the income devel



an idea of the adaptation to the community of this group of patients, provide a perhaps unexpectedly bright picture. It must be remembered, however on reviewing the effects of this disease, that a certain degree of excess mortality — but not significant — has been

shown. It must also be remembered that from a social point of view the effects of this disease were felt to a great extent by many patients. No fewer than every fifth patient had received an invalid pension on account of asthma.

### *Summary*

No systematic socio-medical investigations on the effects of bronchial asthma on the adaptation of the patient to the community appear to have been made previously. A report is made here of a socio-medical study of persons who were still living in Uppsala and its surrounding rural districts after a certain period of time and who had been previously admitted to an Uppsala hospital for the treatment of bronchial asthma. In some parts of the investigation, namely the study of the frequency of changes of residence and the mortality study those who had moved to other districts were also included. 128 patients were interviewed and examined personally by the author. 12 of them had or had had other chronic bronchial diseases, apart from the bronchial asthma, and these patients were therefore not included in the general analyses of the results of studies of different indicators of social adaptation.

Some degree of excess mortality was shown but this was not significant. Of the 16 patients in this series who died (expected number 10.8) it was considered that 5 had died as a direct result of asthmatic attacks. If those cases are disregarded, in which the bronchial

asthma in itself was considered to be the direct cause of death, the disease thus did not appear to have had the indirect effect of increasing the mortality rate. Approximately every fifth patient was receiving an invalid pension solely or mainly by reason of asthma. These patients constituted a group in which both the patient himself and the community were considerably burdened by the social consequences of the disease.

For the purpose of finding out the extent to which these patients moved to other districts a study was made on those who on admission to hospital, were living in the Uppsala urban district. The factors that might be assumed to influence the patients to remain in their own district or move to another were discussed. The standard weighted percentage of those still living in Uppsala after a given period was significantly higher in the asthma series than in the total population. The control material comprised every 36th person in Uppsala these being selected at random from the population schedule.

The general education which was studied in a small number of the patients viz. those in whom the asthma

## IX. GENERAL SUMMARY

The aim of this investigation was to study the long term effect of bronchial asthma on respiratory and circulatory functions and on social adaptation, in relation to the estimated degree of severity of the disease.

The degree of severity was assessed by the approximate number of days of incapacity, days with symptoms, number of hours with asthmatic attacks (attack hours) etc.

Lung volumes, ventilation capacity, physical work capacity and hemodynamic functions were studied during periods in which the condition of the patients as regards their asthma was optimal. Comparisons were made both between different groups, after classification according to the estimated degree of severity of the asthma, and between the series of asthma patients studied and control subjects.

With regard to ventilation capacity the mean intensity of the asthma during the first years almost immediately preceding the test of this function appeared to have a greater influence on the result than the overall degree of severity since the onset of this disease. On the other hand the increase in the ratio of the residual volume (RV) to the total lung capacity (TLC) — a measure of hyperinflation, appears to de-

pend mainly on the degree of severity of the disease as estimated for the whole period since it was first manifested. It is plausible that the reduction in ventilation capacity — which can be demonstrated in free intervals — is to some extent of a reversible nature. Reversibility appeared to be valid to a lesser extent for the state of hyperinflation. It is supposed that hyperinflation — although an at least partly irreversible sign in the present series — does not necessarily imply a state of emphysema (defined as a condition complicated by alveolar wall destruction).

A significant correlation was found for the patients in the present series between ventilation capacity and physical work capacity. The partial correlation between physical work capacity and the estimated degree of severity of the bronchial asthma — when the influence of the reduction of ventilation capacity has been eliminated — was low. This indicates that the influence of the bronchial asthma on the physical work capacity was exerted to the greatest part via the influence on the ventilation capacity. The relationship between the ratio RV/TLC and physical work capacity was found to be of no appreciable importance.

opment from 1943 to 1963 in the employed male persons in the asthma series this was also based on the classifications made by the General Sickness Insurance. Compared with the control material the mean income level of the men in the asthma series appeared if anything to have improved to a somewhat greater extent but this difference was not significant.

It was of especial interest to study the housing standard of the asthma patients, not only because for the majority of people this is related to their income and economic situation but also because it may be generally said that this standard gives some idea of the ambition of a person to organize a satisfactory milieu for himself and his family i.e. his efforts at social adaptation. This study was also of interest in as much as this disease and its course are often said to be associated with the housing standard. The average housing standard of the asthma patients was good. No noteworthy difference was found in this respect between these patients and the total population. Even when the asthma material was divided into sub-series so that the mean standard could be studied in groups that were especially badly affected by the disease e.g. the invalid pensioners, there appeared to be no great difference in housing standard between these groups and the total population. The information with

regard to the total population was obtained from the 1960 population census.

The tobacco-smoking and alcohol consumption habits were studied. There appeared to be good agreement both for the men and the women with regard to the observed and expected numbers of total and almost total abstainers. The expected and observed numbers of non smokers among the women were very similar. With regard to the men a highly significantly smaller number of smokers was found in the asthma series, and the amount of tobacco used by these smokers was less than that used by the smokers in the control material. The expected values for the patients in the asthma series were calculated from information obtained from the City of Uppsala Health Survey of 1961.

The results obtained in the studies of the different indications of adaptation to the community show a perhaps unexpectedly bright picture. It must be pointed out however that in order to obtain an adequate over all impression consideration must be taken of the number of patients — relatively small. It is true — who had died of their asthma. The large average number of days of incapacity and symptom days reported by the patients should also be taken into account as well as the fact that in the group of invalid pensioners in particular the disease had often had deeply felt consequences.

## IX. GENERAL SUMMARY

The aim of this investigation was to study the long term effect of bronchial asthma on respiratory and circulatory functions and on social adaptation, in relation to the estimated degree of severity of the disease.

The degree of severity was assessed by the approximate number of days of incapacity days with symptoms, number of hours with asthmatic attacks ("attack hours") etc.

Lung volumes, ventilation capacity, physical work capacity and hemodynamic functions were studied during periods in which the condition of the patients as regards their asthma was optimal. Comparisons were made both between different groups, after classification according to the estimated degree of severity of the asthma, and between the series of asthma patients studied and control subjects.

With regard to ventilation capacity the mean intensity of the asthma during the five years almost immediately preceding the test of this function appeared to have greater influence on the result than the over all degree of severity since the onset of this disease. On the other hand the increase in the ratio of the residual volume (RV) to the total lung capacity (TLC) a measure of hyperinflation, appears to de-

pend mainly on the degree of severity of the disease as estimated for the whole period since it was first manifested. It is plausible that the reduction in ventilation capacity — which can be demonstrated in free intervals — is to some extent of a reversible nature. Reversibility appeared to be valid to a lesser extent for the state of hyperinflation. It is supposed that hyperinflation — although an at least partly irreversible sign in the present series — does not necessarily imply a state of emphysema (defined as a condition complicated by alveolar wall destruction).

A significant correlation was found for the patients in the present series between ventilation capacity and physical work capacity. The partial correlation between physical work capacity and the estimated degree of severity of the bronchial asthma — when the influence of the reduction of ventilation capacity has been eliminated — was low. This indicates that the influence of the bronchial asthma on the physical work capacity was exerted to the greatest part via the influence on the ventilation capacity. The relationship between the ratio RV/TLC and physical work capacity was found to be of no appreciable importance.

A study was made with the purpose of ascertaining whether certain factors occurring in the case history of these patients had any statistically significant influence on function variables such as the pressure in the pulmonary artery, arterial oxygen saturation etc. The results obtained indicate that the effects of the disease on the pulmonary circulation — demonstrable during symptom free intervals — may be to some extent reversible, i. e. they diminish when the disease becomes less active.

In comparison with the control material the patients in the asthma series showed on an average a relatively strong tendency to an increase in the ratio of the residual volume to the total lung capacity. Since there was no pulmonary hypertension under ordinary resting conditions it may be probable that the increased ratio  $R_V/TLC$  was not caused by extensive destructive pulmonary emphysema. It is more likely that this increase was due only to a less complicated state of hyperinflation. A reasonable conclusion therefore is that bronchial asthma — even if it has previously been intensive for long periods — does not regularly lead to pulmonary hypertension if the disease is not complicated by other conditions which lead to destruction of lung tissue.

In hospital records of patients with a long history of bronchial asthma which during certain periods has been very intensive it is not unusual to find a suggestion and sometimes a definite statement, that so-called cor pulmonale is present. In all of the patients in the present series who underwent

heart catheterization however the resting pressure in the lesser circulation lay within normal values.

No statistically significant excess mortality was shown in the present study. Disregarding the five patients who were considered to have died by direct reason of an asthmatic attack, the observed number of deaths in this series was in agreement with the predicted. It is reasonable to conclude, therefore that uncomplicated bronchial asthma — if death from an acute attack as such can be prevented — does not regularly involve any noteworthy risk of excess mortality by reason of secondary effects on the cardiac and pulmonary functions and the pulmonary circulation.

The results of the socio-medical investigations on this group of patients indicated that their degree of social adaptation was, on an average good. When — for some indicators studied — the asthma series was classified into groups according to the estimated degree of severity of the disease no noteworthy difference was observed between these groups. On assessing the socio-medical consequences of this disease, however several factors should be taken into consideration for instance the relatively large number of days of incapacity due to asthma and days with symptoms and the fact that approximately every fifth patient in this series had received an invalid pension solely or mainly because of asthma. Another important factor is the large demand on hospital care and medical attendance. It was clear that measures of support on the part of the community and private individuals

were of great importance to these patients. The relatively bright picture of the social adaptation of this group, shown in most respects by the results obtained, was probably due essentially to the fact that in practically all of these patients the course of the disease was intermittent. For the twenty year period preceding the present investigation the labour market was on the whole favourable, which must have been of great importance for persons handicapped by bronchial asthma with regard to obtaining work and earning a living. The results of the investigation also appear to indicate that in many of the patients the disease demanded an assiduous manner of living. The fact that for certain of the indicators of social adaptation studied here the present group of patients exhibited a status that to some extent appeared to deviate favourably from that of the control subjects may partly be explained by such a manner of living.

Other important probable reasons for the satisfactory social situation found on the whole in these patients, apart from those mentioned previously are that the effects of the disease on the respiratory and circulatory functions appeared to be at least in some respects, reversible and also that the differences from ordinary respiratory and circulatory conditions, found in this study were not of such a type and degree as to cause permanent severe disablement. The favourable course of the disease — either spontaneous or due to therapy — found in the majority of the patients during the last few years preceding the investigation had made it possible for many to regain the loss in social situation that may previously have been caused by periods of incapacity of varying lengths. The results of the investigation also indicate that the patients had been able to utilize the improvement in their health condition for social stabilization.

## STATISTICAL METHODS

## Notation

$$\text{Mean } \bar{x} = \frac{\sum_{i=1}^n x_i}{n} \text{ where } x_i$$

denotes the  $i$ th recorded value in a series of  $n$  values.

## Relative frequency

$$p = \frac{\text{number of incidences}}{n}$$

## Standard deviation

$$SD = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$$

## Standard error of the mean (SE)

$$SE = \frac{SD}{\sqrt{n}}$$

in comparison between two groups

$$SE_{\bar{x} - \bar{y}} = \sqrt{\frac{\sum (x_i - \bar{x})^2}{(n-1)n} + \frac{\sum (y_i - \bar{y})^2}{(n_y-1)n_y}}$$

## Standard error of a relative freq

$$SE = \sqrt{\frac{p(1-p)}{n}}$$

standard error of difference between two relative frequencies or percentages

$$SE_{p_1 - p_2} = \sqrt{\frac{p_1(1-p_1)}{n_1} + \frac{p_2(1-p_2)}{n_2}}$$

Standard weighted percentages

$$\sum W_j P_j$$

where  $P_j$  is the percentage in the  $j$ th sub-group

$W_j$  is the weight of the  $j$ th sub-group and

$$\sum W = 1$$

Weighted percentages are applied when comparing groups differing in

age distributions. The weights,  $W_j$ , are chosen to correspond approximately to the number of subjects in the respective sub-groups.

## Multiple regression analysis

Linear regression analysis has been performed

A variate,  $y$ , is considered a linear function of  $k$  variables  $x_1, x_2, \dots, x_k$  i.e.

$$y = a + b_1 x_1 + b_2 x_2 + \dots + b_k x_k$$

We calculate the coefficients  $b_1, b_2, \dots, b_k$  according to the least square principle (cf. Snedecor 1956, and Hald, 1948)

## Calculations of expected mortality

In order to compare the observed and expected mortalities, the annual death risks for each individual were first estimated and in the ordinary way consideration was given to sex, age, decade and length of exposure. For this purpose, life tables (Mortality and Length of Life tables) for Sweden were used. For each individual the annual death risks were summated for all the years he was exposed, i.e. between admission and death or admission and withdrawal from the investigation (1961-62). This sum was called  $e$ . If the  $e_i$  values are summated for a patient group the total obtained  $\sum e_i$  = expected number dead. The expected and observed numbers are then compared. For analysis of significance the differences  $o_i - e_i$  can be obtained where  $o_i = 1$  if the  $i$ th person died and  $= 0$  if he had not died. If there

differences are called  $x_i$  then SD and SE can be calculated as given above.

### Significance tests

The following significance tests were used

- 1 In percentage differences  $\chi^2$  test usually with one degree of freedom
- 2 In mean differences

$$CR = \frac{\bar{x} - \bar{y}}{SE_{\bar{x} - \bar{y}}}$$

which approximately follows the standardised normal distribution (or  $t$  test if  $n < 30$ )

- 3 In differences between standard weighted percentages

$$\sum W_i (P_{3i} - P_{7i})$$

a normally distributed critical ratio is produced

$$CR = \frac{\sum W_i (P_{3i} - P_{7i})}{\sqrt{\sum W_i^2 \frac{n_{3i}P_{3i} + n_{7i}P_{7i}}{n_{3i} + n_{7i}} \left(100 - \frac{n_{3i}P_{3i} + n_{7i}P_{7i}}{n_{3i} + n_{7i}}\right) \left(\frac{1}{n_{3i}} + \frac{1}{n_{7i}}\right)}}$$

where  $n_{3i}$  and  $n_{7i}$  correspond to the number of subjects in the compared sub-groups.

- 4 On comparing the observed and the expected number the Poisson distribution has been used as an approximation if the expected number was small
- 5 On comparing the observed and expected mortality the individual differences were first calculated and then

$$CR = \frac{\sum x_i}{\sqrt{n} SE}$$

where  $n$  is the number of individuals and  $x = 0 - e$

The same formula is used when comparing expected and observed demographic and social character

istics (married, over-crowded etc.)

- 6 In analysis of mean rank, the following approximately normally distributed CR was produced

$$CR = \frac{\text{mean rank } \% - 50 \%}{\sqrt{\frac{1}{12n}}}$$

- 7 In regression and correlation analysis tests of coefficients as in Snedecor (1956)

In the significance analyses the patients constituted the unit.

For references on significance tests see Snedecor (1956) and Hald (1948)

### Significance levels

Analyses of significance have been made chiefly in conjunction with the tables. The term significant is used

in accordance with the following convention. If an observed difference between two percentages (or two means) is of such magnitude that the probability  $P$  of obtaining a difference at least equal to the observed value is greater than 0.05 (where the null hypothesis is assumed to hold) the observed difference is said to be non-significant

If  $0.01 < P \leq 0.05$  the difference is said to be (almost) significant and is denoted by

If  $0.001 < P \leq 0.01$  the difference is said to be significant and is denoted by

If  $0.001 \geq P$  the difference is said to be (highly) significant and is denoted by



## Case reports of patients with a chronic disease in addition to the bronchial asthma

Those patients who had or had had another chronic disease — in addition to the bronchial asthma — have been divided into two groups as below

### Group I

Group I comprises patients who were excluded from the general analyses because they had another chronic disease — in addition to the bronchial asthma — which was judged to have had a decisive influence on their life in general state of health and functions

Case No 12. A. J. Male born 1936. Chronic bronchitis + healed pulmonary tuberculosis. Divorced, no children, photographer. Chronic bronchitis since 1952. Only a few years after the onset of bronchitis this was gradually complicated by symptoms of bronchial asthma. About 1934 sanatorium treatment for 18 months for bilateral pulmonary tuberculosis. Repeated follow up examinations showed nothing of note. Right middle lobectomy 1939 for bronchiectasis.

Excluded from the analyses mainly because of chronic bronchitis.

Case No 45. K. J. Female born 1911. Diabetes mellitus + healed pulmonary tuberculosis. Married 1 child, housewife. Onset of bronchial asthma 1945 in which year diabetes mellitus was also manifested, for which continuous insulin treatment had since been given. Had been receiving an invalid pension since 1945 because of asthma. Since the middle of the 1940's had had follow up examinations for suspected tuberculous lung changes. Chest X ray at the time of the present investigation showed left apical changes in the form of patches and streaks, and on the right side small streaky opacities in I 1—I 2.

Excluded mainly because of diabetes mellitus.

Case No 66. G. S. Male born 1904. Chronic

rhinitis. Married, 1 child, ex-painter. Chronic bronchitis since 1932. Only a few years after the onset of chronic bronchitis, this was gradually complicated by symptoms of bronchial asthma. Received invalid pension in 1939 because of bronchial asthma.

Excluded mainly because of chronic bronchitis.

Case No 68. F. J. Female born 1903. Pulmonary sarcoidosis. Unmarried, nulliparous, charwoman. Onset of bronchial asthma 1946. Admitted to hospital 1930 and 1937 for investigation of lung changes which were suspected to be tuberculous but which were later found to be probably sarcoidosis. Mantoux test had never shown a positive result. Chest X-ray at the time of the present investigation revealed large shrunken opacities in the upper regions of both lungs with pleural thickening surrounding the upper parts of the lungs in particular but also the bases, where this was most pronounced on the right side.

Excluded because of pulmonary sarcoidosis.

Case No. 70. S. E. Male born 1903. Healed pulmonary tuberculosis (?). Married, 1 child, typographer. Onset of bronchial asthma 1944. Admitted to hospital at the beginning of the 1930's for investigation of tuberculous-like lung changes. No tubercle bacilli shown. Chest X-ray at the time of the present investigation revealed streaky and patchy to great part calcified, parenchymatous opacities in both apical fields. Bronchography showed an irregular shaped cavity about the size of a grape in the left lower lobe suspected to be tuberculous.

Excluded because of extensive roentgenological lung changes, probably tuberculous.

Case No 77. E. L. Female born 1902. Healed pulmonary tuberculosis. Married, nulliparous, housewife. Onset of bronchial asthma 1944. Pulmonary tuberculosis 1948 (left sided lung process with cavity tubercle bacilli in sputum) for which sanatorium treatment was given for 13 months. (1930 recurrence; sputum once more contained tubercle bacilli) 1948

received invalid pension because of pulmonary tuberculosis. Chest X-ray at the time of the present investigation revealed bilateral tuberculous changes with shrinkage of the left upper lobe in which region cystic emphysematous areas were seen. Pleural thickening in the left apex. Small streaky opacities in the right upper lobe. Upward displacement of the left hilum.

Excluded because of healed pulmonary tuberculosis.

Case No. 82. I. A. Female, born 1901. Mitral valve insufficiency. Married, 1 child, housewife. Onset of bronchial asthma in early childhood. Mitral insufficiency diagnosed in 1932. Diagnosis confirmed by angiography from the left ventricle. Somewhat raised lung capillary pressure. ECG showed signs of left ventricular hypertrophy.

Excluded because of mitral insufficiency.

Case No. 101. L. L. Female, born 1897. Previous cardiac infarction. Married, 1 child, housewife. Onset of bronchial asthma 1942. Probable cardiac infarct 1934 in which year she received invalid pension because of cardiac arteriosclerosis. ECG revealed partial left bundle branch block. Some enlargement of left ventricle seen on roentgenological examination.

During recent years had had increasing symptoms of left cardiac failure.

Excluded because of left cardiac failure.

Case No. 106. K. W. Male born 1896. Chronic myocarditis. Married, 1 child, farmer. Onset of bronchial asthma 1918. Atrial or fibrillation, probably due to myocarditis, diagnosed 1946. Marked general enlargement of the heart found on X-ray. ECG revealed nodular fibrillation and generally low QRS amplitudes.

Excluded because of chronic myocarditis.

Case No. 111. F. L. Female born 1893. Mitral valve insufficiency + angina pectoris. Married, all persons, however. Onset of bronchial asthma 1908. Moderate arterial hypertension known for about 10 years. In 1932 marked physical findings indicated organic disease of the heart, which since this time had been generally enlarged. For the last two or three years the patient had had intermittent precordial pain of angina pectoris type.

Excluded because of mitral insufficiency.

Case No. 112. H. A. Male born 1895. Cerebro-vascular lesion. Widower 4 children, ex farmer. Onset of bronchial asthma 1930. Received an invalid pension in 1948 because of cerebral thrombosis. Diabetes mellitus since 1942, treated with insulin since Cerebro-vascular lesion 1946.

Excluded because of cerebro-vascular lesion and diabetes mellitus.

Case No. 118. J. F. H. L. born 1903. Chronic rheumatoid arthritis. Married, 8 children previously gentleman. Onset of bronchial asthma 1911. Received an invalid pension 1947 because of bronchial asthma. 1930 manifestation of rheumatoid arthritis, which became gradually worse but for many years the joint of the hand and feet. From about 1933 the latter condition rapidly progressed.

Excluded because of chronic rheumatoid arthritis.

To summarize the following diseases influenced the function and general situation of the 12 patients to such a great extent that it was considered that they should be excluded from the general analyses of the investigation results: healed pulmonary tuberculosis (2 patients) organic disease of the heart (2 patients) chronic bronchitis (1 patient) pulmonary sarcoidosis (1 patient) diabetes mellitus (1 patient) cerebro-vascular lesion (1 patient) previous cardiac infarction (1 patient) chronic myocarditis (1 patient) and chronic rheumatoid arthritis (1 patient).

For the 10 patients (case Nos. 12 and 66) with chronic bronchitis, it cannot be stated with certainty whether this was a primary condition or occurred as a complication of bronchial asthma. Both were stated that they had had chronic cough since the beginning of the 1930's, and that they had no other symptoms before this time. Both, however, stated that more marked than symptoms

did not appear until a few years after the onset of chronic bronchitis. Since it could not be precluded with certainty that the chronic bronchitis occurred as a complication of the bronchial asthma some complementary data are given below for these two patients. The figures given first are those for the younger of the two men

PWC<sub>130</sub> 23 % of predicted value, —  
 PWC<sub>150</sub> 25 % of predicted value, —  
 FEV<sub>1.0</sub> 10 % of predicted value, 23 %  
 FEV<sub>2</sub> 51 % of predicted value, 51 %  
 MVV<sub>2</sub> 21 % of predicted value, 26 %  
 RV/TLC 238 % of predicted value, 209 %  
 mean pressure in pulmonary artery at rest  
 12 mm Hg 18 mm Hg  
 mean pressure in pulmonary artery during  
 exercise (300 and 150 kpm/min respectively)  
 35 mm Hg 30 mm Hg  
 cardiac output 5.1 litres/min, 5.5 litres/  
 min.

stroke volume 52 ml, 66 ml.  
 $a-v$  O<sub>2</sub> difference 56 ml/min, 47 ml/min.  
 Housing standard (according to the ranking  
 method, see page 31) 2.0 /s, 15.9 /s.  
 Income at the end of 1963 both were receiving  
 an invalid pension

To summarize it may be said of these two patients that in relation to both the asthma series and the control material they exhibited on the whole a relatively unfavourable status with regard to the above mentioned indices, with the exception however of the values obtained in connection with heart catheterization, where both the pressure and flow conditions were found to lie within normal limits.

## Group 2

Group 2 comprises patients who — in addition to the bronchial asthma — had or had had another disease or suspected signs of another disease. It was not considered in these patients, however, that this supervening disease was of such a type or degree as to have had a decisive influence on their function or general situation. The patients

In this group were therefore included in the general analyses of the results.

Case No. 23. G. I. Female, born 1918. Benign hypertension. Married, 2 children, telephonist. Onset of bronchial asthma 1938. Arterial hypertension since 1930 but there had been long periods when the blood pressure was practically normal. During heart catheterization at the present investigation the blood pressure

140  
 was found to be  $\frac{140}{70}$  95 when no anti-hypertensive

treatment was being given. At the time of these investigations no abnormal signs indicating vascular complications were observed. Neither X-ray examination nor ECG revealed any cardiac abnormalities.

Case No. 34. L. S. Female, born 1914. Previous cardiac infarction? Married, 3 children, housewife. Onset of bronchial asthma 1936. Admitted to hospital 1953 for observation of suspected cardiac infarct. No further cardiac symptoms. At the time of the present investigation neither X-ray nor ECG revealed any pathological cardiac condition.

Case No. 39. M. S. Female, born 1913. Hypertension + hypothyroidism. Married, a hairdresser. Onset of bronchial asthma 1933. Arterial hypertension since about 1935. During the years the blood pressure had varied greatly and during heart catheterization at the present investigation a value of  $\frac{190}{100}$  135

was found in the absence of anti-hypertensive treatment. X-ray examination revealed no cardiac abnormality and the ECG picture was not typical of left ventricular hypertrophy. At the time of the present investigation no abnormal signs of vascular complications were observed and neither were there any signs of hypothyroidism of which the patient was treated at the end of the 1930's.

Case No. 40. M. N. Female, born 1912. Chronic rheumatoid arthritis. Widow, 1 child, charwoman. Onset of bronchial asthma 1911. Rheumatoid arthritis 1936, followed by slight residual symptoms of the hand and foot joints.

Case No. 44. E. L. Male, born 1911. Healed tuberculous infection of tracheobronchial lymph glands. Married, 1 child, electrician.

er Onset of bronchial asthma 1931 Sanator-  
ium treatment 1937 for suspected tubercu-  
losis. Chest X-ray showed sufficient tuberculous  
changes in hilum. Tubercle bacilli not shown.  
Patient discharged as healthy and no follow-  
up examinations made. Chest X-ray at the  
time of the present investigation revealed  
nothing abnormal.

Case No. 64. R. E. Male, born 1906. Previous  
poliomyelitis. Single no children, solicitor's  
clerk. Onset of bronchial asthma 1932. Poli-  
omyelitis at the age of 1 year mainly affecting  
the right leg, which was now slightly short  
and with some muscular atrophy. This pa-  
tient was not included in the calculations of the  
physical work capacity in the present investi-  
gation.

Case No. 78. S. S. M. Male born 1907. Previous  
cardiac infarction. Unmarried. Onset of  
bronchial asthma 1912. Had received in-  
treated pneumonia for bronchial asthma since  
1929. Admitted to hospital 1935 for suspected  
cardiac infarct. The ECG picture at the time of  
the present investigation revealed nothing  
definitely pathological. No definite ECG signs  
of coronary insufficiency were observed dur-  
ing the work test, and there were no definite  
symptoms of angina pectoris either during or  
after this test. X-ray examination revealed no  
cardiac abnormality.

Case No. 87. T. J. Female born 1900. Right  
knee deformed by arthritis. Married, 2 child-  
ren born. Onset of bronchial asthma  
1914. Arthritis of right knee since about 1933,  
and the patient was therefore not included in  
the calculations of the physical work capacity.

Case No. 93. C. C. Female, born 1908.  
Chronic rheumatoid arthritis. Married, 2 child-  
ren, however. Onset of bronchial asthma  
1910. Rheumatoid arthritis since 1931, which  
had slowly progressed, involving mainly the  
knee and hand joints. The lungs were of  
mild type.

Case No. 98. J. K. Female born 1906. Mild  
Parkinson disease. Onset of bronchial asthma  
1925. Mild symptoms of Parkinson disease  
were the end of 1939.

Case No. 99. F. G. Female born 1908.  
Perforation anaemia with previous episodes of  
mild hypothyroidism + mild diabetes mellitus. Mar-  
ried, 1 child, housewife. Onset of bronchial  
asthma 1909. Tubercle bacilli not shown. Chest X-

indicating pernicious myelopathy were ob-  
tained in 1936. At about the same time mild  
diabetes mellitus was manifested, but this did  
not require any special therapy or diet, and  
the blood sugar values had remained within  
normal limits.

Case No. 102. T. M. Female, born 1907.  
Anæmia and high sedimentation rate of un-  
known cause. Married, 1 child, housewife.  
Onset of bronchial asthma 1932. On admission  
to hospital for the present investigation,  
moderate anaemia (111 g) and high  
sedimentation rate (131 mm) were observed.  
Examinations for possible malignancy were  
made, with negative results. This patient is  
represented in the result analyses by those  
values obtained at the out-patient stage of  
these investigations, at which time — 7  
months before admission to hospital — both  
the Hb content and sedimentation rate were  
normal.

Case No. 104. H. P. Female born 1907.  
Previous manio-depressive psychosis. Widow  
1 child. Onset of bronchial asthma 1932. Treated  
in mental hospital on few occasions  
during the 1930's for manio-depressive psy-  
chosis. Operated on in 1927 and 1938 for th-  
yroidosis.

Case No. 107. E. L. Female born 1896.  
Healed pulmonary tuberculosis. Unmarried,  
nulliparous, ex-nurse. Onset of bronchial  
asthma 1906. In 1917 chest X-ray revealed  
patch on the right lung, diagnosed tuber-  
culosis. Sanatorium treatment given for about  
5 months. Chest X-ray at the time of the  
present investigation showed streaky and  
patchy right apical opacities, otherwise nothing  
abnormal.

Case No. 113. V. V. Male born 1893. Gastro-  
duodenal resection + resection of colon. Mar-  
ried, 2 children, previously mill filter. Onset  
of bronchial asthma 1910. On gastro-duodenal  
resection + resection of colon in 1934 for large  
cancer of stomach infiltrating into the meso-  
colon. Subsequent course free of complica-  
tions.

Case No. 120. C. J. Male, born 1891. Healed  
pulmonary tuberculosis. Widower, 2 children,  
previously wood-worker. Onset of bronchial  
asthma 1908. In about 1925 lung changes were  
seen at both apices suspected to be tubercu-  
lous. Tubercle bacilli never shown. Chest X-

ray at the time of the present investigation showed occasional calcifications in both apices and a few calcifications in the middle part of the right lung. No signs of active tuberculosis.

*Patients with a relatively large deviation from normal values with regard to basal metabolic rate and hemoglobin content*

Apart from the conditions present in the patients of groups 1 and 2, described above, no other signs of supervening diseases were observed during these investigations. A brief report will be made below however of those cases where relatively large deviations from normal values were found for basal metabolic rate and hemoglobin concentration.

A basal metabolic rate exceeding  $+20\%$  of the predicted value (prediction according to sex, age, height and body weight [Harris and

Benedict, 1919]) was recorded for the following patients: Case No. 97 ( $21\frac{1}{4}\%$ ) No. 85 ( $22\%$ ) No. 20 ( $22\frac{1}{2}\%$ ) No. 78 ( $26\frac{1}{2}\%$ ) No. 115 ( $28\%$ ) No. 110 ( $31\frac{1}{2}\%$ ) No. 113 ( $31\frac{1}{2}\%$ ) No. 81 ( $36\frac{1}{2}\%$ ) No. 88 ( $37\%$ ) No. 116 ( $35\%$ ) No. 114 ( $47\%$ ). A basal metabolic rate of less than  $-20\%$  of the predicted value was noted for case No. 43 ( $-23\%$ ).

In none of these patients nor in any of the other patients in the asthma series was there any reason to suspect current thyrotoxicosis or myxoedema.

During the investigations the following women were found to have a hemoglobin concentration of less than 11.5 gram/100 ml blood: Case No. 30 ( $11.3\text{ g}\%$ ) No. 29 ( $10.6\text{ g}\%$ ) and No. 57 ( $10.5\text{ g}\%$ ).

One of the men, case No. 72, was found to have a hemoglobin concentration of less than  $12.5\text{ g}/100\text{ ml}$  blood, i.e.  $11.7\text{ g}\%$ .

No signs or symptoms indicating cardiac or pulmonary or other diseases, apart from those mentioned were found in this series of patients.

## Key to the ward case records

Case No.	Case record No.	Case No.	Case record No.	Case No.	Case record No.
1	2136/02	44	718/02	87	1189
2	2031/02	45	1110/02	88	0035/02
3	714/02	46	307/02	89	2712/02
4	331/02	47	452/00	90	344/02
	3722/02	48	322/10	91	1210/02
6	2161/02	49	111/02	92	1808/02
7	2302/02	50	222/02	93	1121/02
8	2311/02	51	1071/02	94	2100/02
9	2048/02	52	107/02	95	2130/02
10	2037/02	53	1808/02	96	2170/02
11	2224/02	54	480/02	97	012/10
12	1002/02	55	1107/02	98	1187/02
13	2070/02	56	3070/02	99	1102/02
14	2230/02	57	304/02	100	202/10
15	1091/02	58	007/02	101	1122/02
16	1192/02	59	472/02	102	005/02
17	2037/02	60	110/02	103	2261/02
18	910/02	61	1222/02	104	2922/02
19	217/02	62	304/02	105	1111/02
20	424/02	63	222/02	106	2224/02
21	2112/02	64	2202/02	107	702/02
22	022/02	65	2702/02	108	700/02
23	2164/02	66	220/02	109	1001/02
24	2012/02	67	2100/02	110	1061/02
25	004/02	68	10/02	111	2000/02
26	200/02	69	200/02	112	2002/01
27	2121/02	70	402/02	113	2422/02
28	2007/02	71	207/02	114	1222/02
29	722/02	72	2001/02	115	1002/02
30	722/02	73	200/02	116	1000/02
31	272/02	74	200/02	117	2717/02
32	212/02	75	1070/02	118	1002/02
33	000/02	76	224/02	119	221/02
34	102/02	77	021/02	120	1002/02
35	202/02	78	221/02	121	1002/02
36	112/02	79	000/02	122	1002/02
37	1120/10	80	220/02	123	1002/02
38	212/02	81	170/02	124	222/02
39	101/02	82	100/02	125	221/20
40	1122/02	83	2020/02	126	2027/02
41	1100/02	84	2100/02	127	1002/02
42	212/02	85	200/10	128	992/02
43	010/02	86	021/02		

the records of the Medical Clinic, University Hospital, L'puala.

case record of the Chest Clinic, University Hospital, L'puala.

case record of the Samaritan Hospital, L'puala.

# REFERENCES

- AAS, K. Prognosis for asthmatic children  
*Acta Paed Scand* 140:87 1963.
- ASBOTT O A HOPKINS, W. A., VAN FLAT, W.  
L. and ROBINSON S. A new approach to  
pulmonary emphysema. *Thorax* 8 116,  
1953
- ALEXANDER H L: Proceedings of the third  
international congress of allergology, p.  
109 Plasmation, Paris 1958.
- AMERICAN THORACIC SOCIETY Chronic bronch-  
itis, asthma and pulmonary emphysema.  
*Am J Res Dis* 83 762 1962.
- ANDERSSON, H. and BACKLUND L. Estimation  
of basal oxygen uptake. *Scand Med* 63 734  
1961
- ÅKE LEMARK, E. Life death and disease in  
Sweden. Almqvist & Wiksell Stockholm,  
1960
- ASMUSEN E. and NIELSEN M: Cardiac output  
in rest and work at low and high oxygen  
pressures. *Acta Physiol Scand* 33 3 1955.
- ÅSTRAND, JENA Aerobic work capacity in men  
and women with special reference to age  
*Acta Physiol Scand* 31 ppl 109 1960
- ÅSTRAND, P-O Experimental studies of phys-  
ical working capacity in relation to sex and  
age Ejnar Munksgaard, Copenhagen, 1952
- BALDWIN ELEANOR DE F: Bronchial asthma  
*Inner J Med* 1:180 1916.
- BALDWIN ELEANOR DE F COURNAND A and  
DICKINSON W R: Pulmonary Inefficiency  
*Med* 28 201 1949
- BARKER, S. B. and SUMMERSON W. H. The  
colorimetric determination of lactic acid in  
biological material. *J Biol Chem* 138:833  
1941
- BATES, D V, KNOTT J M G and CURRIE,  
R V. Respiratory function in emphysema  
in relation to prognosis. *Quart J Med* 25  
13 1956.
- BEALE, H V FOWLER, W. S. and CONNOR,  
J H. Pulmonary function studies in 20  
asthmatic patients in the symptom free  
interval. *J U* 23 1 1952.
- BENNETT, R. H. WILLIAMS, J. F. and WHITE,  
D. H. The effect of acetylcholine infusion  
upon cardiac dynamics in patients with  
pulmonary emphysema. *Inner Res Resp  
Dis* 87 57 1963.
- BENSTEN B G Forekomst og invaliditet ved  
astma - bronkitt - emfysem. *Tidsskr for  
Det Norske lægeforening* 16 162 1962
- BENGLUND E., DIRATH G, BJURZ, J GADNRY  
G KJELLMER, I SANDQVIST L. and DOBER-  
HOLM, B. Spirometric studies in normal  
subjects. I. Forced expirations in subjects  
between 7 and 70 years of age. *Acta Med  
Scand* 173 183 1963.
- BRANSTEN L, D'SILVA, J L. and BLENDL D.  
The effect of the rate of breathing on the  
maximum breathing capacity determined  
with a new spirometer. *Thorax* 7 103 1952.
- BEVINGOLD A HOLMGAARD A and JONSSON B.  
The effect of body posture on the circula-  
tion at rest and during exercise with spe-  
cial reference to the influence on the tro-  
chanteric. *Acta Physiol Scand* 49 279 1960.
- DIRATH, G, KJELLMER, I and SANDQVIST L.  
Spirometric studies in normal subjects. II.  
Ventilatory capacity test in men. *Acta  
Med Scand* 173 193 1963.
- BOLT W VALENTIN H. and TRETZ, V. Drucke  
in der Pulmonalarterie Herzinhalt  
nieren und Alveolarpartialdruck bei  
respiratorischer Hypoxie in steigender Höhe  
bis zu 4000 und 5000 M über Meeresspiegel.  
Per. vonden. Arch Kreisf Forsch 2 19 1937

- BORDEN, C. W. WILSON R. H., LARRY R. V. and WELLS, H. S. Pulmonary hypertension in chronic pulmonary emphysema. *Amer J Med* 8 701, 1950.
- BOWEN, G. Deaths and illness from bronchitis emphysema and thoma. *Amer Rev Resp Dis* 83 894 1961
- BRUCE, T. DANLSTROM, G. and UGGLA L.-G. Sociomedical studies of tuberculous patients from Stockholm. The social structure of the material. *Acta Tuberc Scand Suppl* 50 9 1960.
- CARDILL, R. S. and BRUCE PEARSON, R. S. Death in rheumatic fever 14 341, 1959
- CRAIGHEAD SYMPTOM Terminology definitions and classification of chronic pulmonary emphysema and related conditions. *Thorax* 14 396, 1959
- CLAUSSEN, O. Asthma Norge. *Nord Med* 37 855 1918.
- COTTE, J. E. PISA, Z. and THOMAS, A. J. Effect of breathing oxygen upon cardiac output, heart rate, ventilation, systemic and pulmonary blood pressure in patients with chronic lung disease. *Clin Sci* 25 303, 1963.
- DANLSTROM, G. Sindemjuka fruktambet. *Nord Med* 8 891 1912
- DANLSTROM, G. Studies of the physical working capacity in pulmonary tuberculosis. *Nord Med* 83 417 1953.
- DANLSTROM, G. Physical working capacity and electrocardiogram in pulmonary diseases. *Acta Med Scand* 21 228, 1957
- DIXON, L. WHITTAKER, J. L. GOSLIN, B. LEWIS, B. M. H. FRY, F. W. and SMITH, R. J. *Proc Assoc Amer Phys* 64 226, 1931
- DONALD, K. W. BRINOT, J. M. and WADE, G. L. A study of minute-to-minute changes of thoracic oxygen content during oxygen uptake and cardiac output and its effect on the rate of steady state during exercise in rheumatic heart disease. *J Clin Invest* 33 1146, 1954
- EKLUND, B. V. Fatal bronchial asthma. *Thorax* 8 143 1957
- FALL, M. U. L. Untersuchungen über die Fruchtbarkeit der verschiedenen Gruppen von Gebärenden. *Arch Psychiatr Nervenheilkd* 191 1, 1953
- FAGERBERG, E. Studies in bronchial asthma. *Acta Allerg* 11 327 1957
- FISHMAN, A. P. McCLEMENT J. HUNDELSTY, A. and COURMAND, A. Effect of cut anoxia on the circulatory and respiration in patients with chronic pulmonary disease studied during the steady state. *J Clin Invest* 31 770, 1952.
- FISHMAN, A. P. FRITTS, H. W. and COURMAND, A. Effects of acute hypoxia and exercise on the pulmonary circulation. *Circulation* 23 304 1960
- Effect of breathing carbon dioxide upon the pulmonary circulation. *Circulation* 23 226 1960
- FLETCHER, C. M., HOBBS-JONES, H. McNICOL, M. W. and PRUDEN, V. E. The diagnosis of pulmonary emphysema in the presence of chronic bronchitis. *Quart J Med* 32 33, 1963.
- FOVLER, V. O., WESTCOTT R. V. SCOTT R. C. and HUNT, L. M. The cardiac output in chronic cor pulmonale. *Circulation* 8 888, 1953.
- FRITTS, H. W. HARRIS, P. CLAUSS, R. H. ODELL, J. E. and COURMAND, A. The effect of tyboline on the human pulmonary circulation under normal and hypoxic conditions. *J Clin Invest* 37 99 1958.
- GARDNER, E. A. Evaluation of pulmonary function. Results in chronic obstructive lung disease. *Ann Res Med* 13 319 1962
- GARY, L. and TULLIE, J.-C. The amount of trapped plasma in high speed microcapillary hematocrit centrifuge. *Scand J Clin & Lab Invest* 13 642, 1961.
- GLOOR, F. Zur Pathologie des Asthma bronchiale. *Schweiz Arch Pathol Anat* 223 189 1934.
- GOUGH, J. Discussion on the diagnosis of pulmonary emphysema. *Proc Roy Soc Med* 45 376 1952.
- GRAM, H. C. Asthma bronchiale. *Nord Med* 37 816, 1918
- GRANT, A. J., JOHNSON, B. and STRANDER, T. Studies on the central circulation at rest and during exercise in the supine and sitting body positions in old men. *Acta Med Scand* 168 123, 1961.
- GROSS, G. and HÖRSTHOFF, B. Hydrometrie



- WALKUP H E. and CONNOLLY ELIZABETH C.: The dimensions of the chronic respiratory disease problem *Amer J Publ Health Suppl* 53 1963
- WESTCOTT R V, FOWLER, N O, SCOTT H C., HAUSSTEIN V D and MCGUIRE, J: Anoxia and human pulmonary vascular resistance *J Clin Invest* 30:937 1951
- WILANDER, O: Blood volume determinations in surgical practice. *Acta Chir Scand Suppl* 208, 1950
- WILLIAMS, D A: Social importance of allergic diseases. Premier Congrès International d'Allergie Zürich 1951 p. 42 Karger Basel.
- WILLIAMS, D A: Deaths from asthma in England and Wales. *Thorax* 8 137 1953.
- WILLIAMS, D A. and LEOPOLD J G.: Death from bronchial asthma III Congr International d'Allergologie Paris 1958. *Acta All* 14 83 1959
- WHITAKER, W: Pulmonary hypertension in congestive heart failure complicating chronic lung disease *Quart J Med* 23 37 1954
- YU P V., LOVEJOY F W, JOOS, H A., YUK, R. E. and MCCANN W S: Studies of pulmonary hypertension. *J Clin Invest* 32 130 1953.

## ACKNOWLEDGEMENTS

This study which was carried out during the years 1961-64 was originally suggested by Professor Erik Ask-Upmark, head of the Department of Internal Medicine University Hospital, Uppsala under whose guidance I have had the privilege of working. I am greatly indebted to him for his valuable advice and the continuous interest which he has shown my work. Professor Ask-Upmark supervised the patients in this study and his extensive knowledge of bronchial asthma has been inestimable to me.

Professor Ragnar Berenstam—under whom I am working at present—head of the Department of Social Medicine, University Hospital, and Professor Gunnar Strom, head of the Department of Clinical Physiology University Hospital, who generously placed their departments at my disposal—both participated in the original planning of this study. Their great interest, encouraging guidance and constructive criticism throughout the course of the investigation, have been of great importance and I would like to express my sincere gratitude and appreciation which I feel toward them.

Docent H. Werner has given me constant support from the beginning of the work. His great interest, helpful and timely criticism during the course of the study has been of great value to me and I wish to express my sincere thanks.

I would also like to express my appreciation to Docent Gunnar Ekblad for his great help with the statistical analyses. I thank him for his unflinching interest in my work and for innumerable profitable discussions.

Professor Erik Knutsson, head of the Department of Diagnostic Radiology

University Hospital, and Docent Herman Lodin generously placed their laboratory at my disposal and gave me valuable help in judging the X-ray films. All heart volumes were calculated with the aid of Doctor Björn Carlsson to whom I express my thanks.

Professor Erik Hedvall, former head of the Chest Department University Hospital, Professor Arn Sjöberg, head of the Department of Otolaryngology University Hospital, Docent Runar Brenning, head of the Medical Department of the Samaritan Hospital, Uppsala, and Doctor Erik Fagerberg, consultant allergist at University Hospital, have kindly placed at my disposal information about patients who are or have been under their supervision.

I should like to acknowledge the great help which I have received from the staff of the Departments of Internal Medicine, Clinical Physiology, Social Medicine and Diagnostic Radiology and the Chest Clinic, University Hospital.

I express my thanks to Miss Birgitta Keredal for invaluable help in the analyses of blood and gas samples and also to Mrs F. G. Graf for help with figure drawings.

The work has been translated by Mrs Maud Marsten. I wish to thank her for helpful co-operation.

I would like to express my appreciation to the Swedish National Association for Heart and Chest Diseases and to the Medical Faculty of the University of Uppsala for the generous grants made to defray the costs of my investigation.

To all others who have in various ways made it possible for me to carry out this work, I extend my sincere thanks.

Lars Irnell



# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 418

## EXPERIMENTAL URAEMIC PULMONARY OEDEMA

including: criteria for pulmonary oedema  
in septic rabbits, the role of uraemia and overhydration,  
and a literary survey on the problems  
of uraemic pulmonary oedema (fluid-retention lung, etc.)

by

BENGT LINDQVIST

*Acta medica* Vol. 176

---

LUND 1966



# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 418

## EXPERIMENTAL URAEMIC PULMONARY OEDEMA

including: criteria for pulmonary oedema  
in anuric rabbits, the role of uraemia and overhydration,  
and a literary survey on the problems  
of uraemic pulmonary oedema (fluid-retention lung, etc.)

by

BENGT LINDQVIST

---

*Accompanies Vol. 176*

LUND 1964



ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 418

FROM THE MEDICAL CLINIC B (RENAL CLINIC),  
UNIVERSITY OF LUND, LUND, SWEDEN

# EXPERIMENTAL URAEMIC PULMONARY OEDEMA

including: criteria for pulmonary oedema  
in anuric rabbits, the role of uraemia and overhydration,  
and a literary survey on the problems  
of uraemic pulmonary oedema (fluid-retention lung, etc.)

BY

BENGT LINDQVIST

LUND 1964



# ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of *Nordiskt Medicinskt Arkiv* founded in 1869 by Axel Key. The first volume of *Acta Medica Scandinavica* is therefore numbered LII (52).

The chief editors have been Axel Key 1869—1900, C. G. Santesson 1901—1915, I. Holmgren 1916—1957 and Birger Strandell 1958 to date.

*Acta Medica Scandinavica* publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board. Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the Editor at the address below.

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that if accepted it will not be republished in any other journal in the same or similar form without the Editor's written consent.

Papers will be published in English, French or German at the authors' choice, followed by a summary in English.

The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left hand margin.

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author. No paper shall exceed 24 printed pages.

## Subscription

The annual subscription to the journal, covering two volumes, each of 6 numbers, is 140 Sw. crowns or U.S. \$ 27.25 including postage in the Scandinavian countries and in Holland 120 Sw. crowns.

*Address for subscriptions and all communications*

ACTA MEDICA SCANDINAVICA

P O Box 2052 Stockholm 2

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 418

FROM THE MEDICAL CLINIC B (RENAL CLINIC),  
UNIVERSITY OF LUND, LUND, SWEDEN

# EXPERIMENTAL URAEMIC PULMONARY OEDEMA

including criteria for pulmonary oedema  
in anuric rabbits, the role of uraemia and overhydration,  
and a literary survey on the problems  
of uraemic pulmonary oedema (fluid-retention lung etc.)

BY

BENGT LINDQVIST

LUND 1961

# ACTA MEDICA SCANDINAVICA

has been published since 1910 as a continuation of *Nordiskt Medicinskt Arkiv* founded in 1869 by Axel Key. The first volume of *Acta Medica Scandinavica* is therefore numbered LII (52).

The chief editors have been Axel Key 1869—1900, C. G. Saniésson 1901—1915, I. Holmgren 1916—1957 and Birger Strandell 1958 to date.

*Acta Medica Scandinavica* publishes original research papers in the field of internal medicine. Priority will be given to authors from countries which are represented on the editorial board. Contributors from those countries should submit their papers to a representative of the country in question. Contributors from other countries should send their papers to the Editor at the address below.

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that if accepted it will not be republished in any other journal in the same or similar form without the Editor's written consent.

Papers will be published in English, French or German at the authors' choice, followed by a summary in English.

The manuscripts shall be in final form as submitted. They shall be typewritten with double spacing and broad left hand margin.

If a paper exceeds 16 printed pages, the cost for the excess pages shall be borne by the author. No paper shall exceed 24 printed pages.

## Subscription

The annual subscription to the journal covering two volumes, each of 6 numbers, is 140 Sw. crowns or U.S. \$ 27.25 including postage in the Scandinavian countries and in Holland 120 Sw. crowns.

*Address for subscriptions and all communications*

ACTA MEDICA SCANDINAVICA  
P O Box 2052 Stockholm 2

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

# Contents

<i>Introduction</i>	7
<i>Chapter 1 A short historical survey on pulmonary oedema with special reference to renal disease</i>	9
<i>Chapter 2. Literary survey on the problems of uraemic pulmonary oedema</i>	11
Clinical picture 11 — Microscopical picture 11 — Radiographic abnormalities 12 — Pathogenesis 12	
<i>Chapter 3. Literary survey of experimental uraemic pulmonary oedema</i>	18
<i>Chapter 4. Technique</i>	20
Appearance and behaviour of the animals 20 — Respiratory rate 21 — Heart-rate and electrocardiograms 21 — Chest radiography 21 — Gross observation at autopsy 22 — Prothly fluid in the air ways 24 — Lung weight 24 — Water content of the lung 25 — Microscopical studies 27 — Lung volume 27 — Diagnosis of pulmonary oedema 28	
<i>Chapter 5. Control material</i>	29
Examination of the living animals 29 — Examination immediately after death of blood animals 31 — Examination after death of animals dying spontaneously without rigor mortis 37 — Examination 18 hours after death of animals with rigor mortis 38 — Pulmonary oedema and uraemia 40	
<i>Chapter 6. Symptoms and pulmonary oedema in overhydration</i>	41
Increase of blood-volume 41	
Blood transfusion 41	
Own investigations 42	
Symptoms in 1: 42 — Postmortem findings 43 — Criteria of pulmonary oedema 45 — Pulmonary oedema and uraemia 45	
Infusion of dextran 45	
Own investigations 45	
Symptoms in 1: 47 — Postmortem findings 51 — Criteria of pulmonary 52 — Pulmonary oedema and uraemia 53	
Increase of the extracellular fluid 53	

*Translated by Mrs Greta Sargeant*

*Printed in Sweden*

LUND 1964

BERLINGSKA BOKTRYCKERIET

## Introduction

In a medical renal clinic, which admits many patients with severe renal failure, pulmonary changes of the type here designated as uraemic pulmonary oedema are of great diagnostic and therapeutic importance. In close co-operation with the department of diagnostic radiography at the University clinics extensive studies has been carried out at this clinic in the last 20 years. Uraemic pulmonary oedema is a common complication of severe renal disease. Alwall & Kjellstrand, in a survey of 903 patients with an acute or chronic renal failure, found radiographic pulmonary changes of this type in 42.7 % of the cases.

The diagnosis of uraemic pulmonary oedema is based mainly on the X ray findings. Clinically the symptoms may at first be insignificant, even when the radiographic changes are considerable. Alwall and Kjellstrand point out that the problem of differentiation can make a correct *in vivo*-diagnosis difficult. They stress that the diagnosis is a radiographic-clinical one. The radiographic and the clinical pictures of uraemic pulmonary oedema can be complicated by such factors as ab-

1 raised dia

phragm in intestinal paralysis, accumulation of secretion in the respiratory tract, atelectasis, bronchitis and bronchopneumonia hyperaemia, and pleural effusion, etc. Moreover many patients with uraemic pulmonary oedema in addition to their uraemia and pulmonary changes are severely ill from other causes (for instance traumatic injuries, infections and shock). For this reason they are more or less unable to co-operate at clinical examinations and the X ray examinations must be made at the bedside. At autopsy final and postmortal changes may make it difficult to draw definite conclusions as regards the changes that were present in their lungs *in vivo*. The variety of names used in the literature for these pulmonary changes (uraemic pulmonary oedema, fluid retention lung, uraemic pneumonitis, congestive heart failure, etc.) reflect the different concept of the pathogenesis.

The problems connected with the differential diagnosis and the patients inability to co-operate are the main factors that hamper studies of uraemic pulmonary oedema *in vivo*. I have therefore tried to throw some light on



## A short historical survey on pulmonary oedema with special reference to renal diseases

*Pulmonary oedema. An effusion of serous fluid into the air vesicles and interstitial tissue of the lungs.*  
(Dorland 1937.)

In about 1500 B.C. oedema and dyspnoea were for the first time mentioned in writing on papyrus from ancient Egypt (93). The first clinical description on pulmonary oedema in our times was given by Maloët in 1752. Surveys on pulmonary oedema were published by several authors (3, 43, 48, 121, 157, 168, 173, 217, 248, and others).

Pulmonary oedema has been described in cardiac, pulmonary cerebral, and renal diseases in hypertension, infections, burns, intoxications, liver cirrhosis, and other conditions.

Pulmonary oedema in renal disease was first described by Legendre (1864) and Frerichs (1851) who observed pulmonary oedema in patients with nephritis. Goodhart (1879) reported pulmonary oedema in 5 children with acute renal insufficiency. He recommended large doses of laxatives to control oedema, as digitalis usually had no great effect. Telsaler (1000) and Merklen (1000) found that pulmonary oedema is common in nephroderoids and chronic glomerulonephritis but uncommon in subacute glomerulonephritis with nephrosis. Telsaler (1000) and Whitehall (1930) observed an increased tendency to pulmonary oedema

in increasing renal insufficiency. Cameron (1918) noted pulmonary oedema in 74 % at autopsy of 50 cases with chronic nephritis. Alwall & Herner (1949) showed that pulmonary oedema together with uraemia are the greatest risks to a patient suffering from acute renal failure, and that the risks of oedema in acute nephritis with oliguria are of great practical importance.

Bluemle et al. (1959) on the basis of clinical symptoms, reported an incidence of congestive failure in 100 cases of acute tubular necrosis of 15—32 %. Alwall (1960) in an account of the results of systematic X-ray investigations, reported changes of the fluid retention lung type in 280 out of 600 patients (43 %) with renal insufficiency. 66 % had acute tubular necrosis. Neth (1961) noted cardiac insufficiency and pulmonary oedema clinically or radiologically in 9 % of his cases with acute or subacute glomerulonephritis.

Langendorf & Pick (1938) published a survey of cardiac failure in acute nephritis. Ellis (1936) noted cardiac insufficiency in 20 % and Whitehall (1939) found it in 70 % of such cases. The symptoms were cardiac enlarge-



the diagnostic and pathogenetic aspects of this clinically important question by experiments on animals

A survey of the numerous reports of experimental studies in animals shows, however that even the definition of pulmonary oedema and the criteria for the diagnosis are subject to great uncertainty. In view of the different opinions as regards the concept of

uraemic pulmonary oedema, clinically as well as experimentally these experiments were designed to study particularly the diagnostic value of the criteria in pulmonary developed under varying experimental conditions. The question of uraemia and the significance of overhydration in uraemic pulmonary oedema has also been studied by experiments in animals

## A short historical survey on pulmonary oedema with special reference to renal diseases

*Pulmonary oedema:* An effusion of serous fluid into the air vesicles and interstitial tissue of the lungs.

(Dorland 1947)

In about 1500 B.C. oedema and dyspnoea were for the first time mentioned in writing on papyrus from ancient Egypt (95). The first clinical description on pulmonary oedema in our times was given by Maloët in 1752. Surveys on pulmonary oedema were published by several authors (3, 43, 48, 121, 157, 168, 178, 217, 246 and others).

Pulmonary oedema has been described in cardiac, pulmonary, cerebral, and renal diseases, in hypertension, infections, burns, intoxications, liver cirrhosis, and other conditions.

Pulmonary oedema in renal disease was first described by Legendre (1864) and Frerichs (1851) who observed pulmonary oedema in patients with nephritis. Goodhart (1879) reported pulmonary oedema in 5 children with acute renal insufficiency. He recommended large doses of laxatives to control oedema, as digitalis usually had no great effect. Teissler (1900) and Merklen (1900) found that pulmonary oedema is common in nephrosclerosis and chronic glomerulonephritis but uncommon in subacute glomerulonephritis with nephrosis. Teissler (1900) and Whitehall (1939) observed an increased tendency to pulmonary oede-

ma in increasing renal insufficiency. Cameron (1948) noted pulmonary oedema in 74 % at autopsy of 50 cases with chronic nephritis. Atwall & Herner (1949) showed that pulmonary oedema together with uraemia are the greatest risks to a patient suffering from acute renal failure, and that the risks of oedema in acute nephritis with oliguria are of great practical importance.

Bluemle et al (1959) on the basis of clinical symptoms reported an incidence of congestive failure in 100 cases of acute tubular necrosis of 15—32 %. Atwall (1960) in an account of the results of systematic X-ray investigations, reported changes of the fluid retention lung type in 260 out of 607 patients (43 %) with renal insufficiency. 60 % had acute tubular necrosis. Nieh (1961) noted cardiac insufficiency and pulmonary oedema clinically or radiologically in 9 % of his cases with acute or subacute glomerulonephritis.

Langendorf & Pick (1938) published a survey of cardiac failure in acute nephritis. Ellis (1936) noted cardiac insufficiency in 20 % and Whitehall (1939) found it in 70 % of such cases. The symptoms were cardiac enlarge-

ment hypertension dyspnoea and raised venous pressure

X ray in the diagnosis of pulmonary oedema in renal disease was first used by Ehrlich & McIntosh (1932) but Zdansky (1933) and Roubier & Plachu (1934) were among the first to describe the more characteristic features. Later numerous authors have reported results and discussed the X ray findings and their pathogenesis (6 10 11 16 20 21 26 31 32 38 39 43 54 60 63 64 65 91 103 105 118 120 122 123 125 126 131 132 136, 140 144 145 157 160 166 171 193 194 197 198 205 215 216 222 227 239 251 259 261 262)

The radiographic changes characteristic of pulmonary oedema in patients with renal disease have been called by many names, because of the varying views held on their pathogenesis. Some authors have used unspecific designations, such as "pulmonary oedema" (122 125) "acute pulmonary oedema" (261) "chronic pulmonary oedema" (194 251). Among the most usual designations are "pul-

monary oedema in renal insufficiency "pulmonary oedema in uraemia" "uraemic oedema of the lungs" and "uraemic pulmonary oedema" (38 60 63 105 145 222). Some talk about "pulmonary oedema of renal origin" "nephrogenic pulmonary oedema" (131 144 160) others about "uraemic lung" "uraemic pneumonitis" "uraemic pneumonia" "pulmonary changes in uraemia" "pulmonary manifestations of azotaemia" (26 118 120 123 126 140 259 262) and of "myocardial failure in nephritis" "heart failure in acute nephritis" "heart failure and pulmonary oedema" "pulmonary congestion in uraemia" (54 60 196 253). Other designations are "pulmonary changes in hypertension" "pulmonary changes in nephritis" (124 205).

Eventually the terms "fluid lung" (13 16 17) "fluid retention lung" (21) and "uraemic fluid lung" (244) were introduced.

In this survey the neutral designation "uraemic pulmonary oedema" will be used.

## Literary survey on the problems of uraemic pulmonary oedema

The clinical picture of clinically manifest severe uraemic pulmonary oedema resembles that of pulmonary oedema of other origin (59-91 and others). The auscultatory findings, on the other hand, may be minimal even in cases of relatively extensive radiographic changes (13, 32, 63). Pleural effusion is present in small or moderate amounts in about 50 % of the cases (59). Facial oedema is common, especially in cases of acute renal insufficiency whereas oedema of the legs is rare (20). The patients exhibit no or only slight dyspnoea and no cyanosis. Tachypnoea occurs but its correlation with the degree of pulmonary oedema is relatively poor. In patients with uraemic pulmonary oedema, alveolar ventilation is reduced and diffusely distributed among all parts of the lung, and vital capacity declines (92). Fever is not a clinical feature (32). Tachycardia is common (11, 125). Moderate anaemia and a slight decrease of serum proteins are usual features. In most cases the patients have hypertension of varying degrees, but pulmonary changes can also occur in patients who have a low blood pressure or are in shock (3, 11, 18, 20, 31, 196). The hypertension is usually reduced or

disappears after the pulmonary oedema has been brought under control by dehydration. Occasionally however the blood pressure will rise in response to removal of the oedema (3). Many overhydrated patients, with or without oedema of the lungs, show signs of cerebral oedema, such as restlessness, personality changes, mental confusion, and convulsions (20, 21). Ascites does not seem to be a common feature.

Uraemic pulmonary oedema in patients can be complicated by bronchitis and stagnation of secretion, atelectasis, bronchopneumonia, haemorrhages, etc., and, notably in nephrosclerosis and chronic glomerulonephritis, also by congestion as a result of cardiac insufficiency (11, 18, 20, 21, 81, 92, 102, 105, 163, 213).

The microscopic picture of uraemic pulmonary oedema at autopsy has been described by several authors (31, 32, 63, 126 and others) who observed hyperaemia in the lung capillaries, thickening of the alveolar septa, fibrinous or albuminous alveolitis with partial hyalinisation of the intra alveolar exudate, and hyaline membranes clothing the alveolar walls. Small haemorrhages intra alveolarly and intersti-

ment, hypertension, dyspnoea and raised venous pressure.

X ray in the diagnosis of pulmonary oedema in renal disease was first used by Ehrich & McIntosh (1932) but Zdanaky (1933) and Roubier & Plachn (1934) were among the first to describe the more characteristic features. Later numerous authors have reported results and discussed the X ray findings and their pathogenesis (6 10 11 16 20 21 26 31 32 38 39 43 54 60 63 64 65 91 103 105 118 120 122 123 125 126 131 132 136 140 144 145 157 160 166 171 193 194 197 198 205 215 216 222 227 239 251 259 261 262)

The radiographic changes characteristic of pulmonary oedema in patients with renal disease have been called by many names, because of the varying views held on their pathogenesis. Some authors have used no specific designations, such as "pulmonary oedema" (122 125) "acute pulmonary oedema" (261) "chronic pulmonary oedema" (194 251) Among the most usual designations are "pul-

monary oedema in renal insufficiency" "pulmonary oedema in uraemia" "uraemic oedema of the lungs" and "uraemic pulmonary oedema" (38 60 63 105 145 222) Some talk about "pulmonary oedema of renal origin" "nephrogenic pulmonary oedema" (131 144 160) others about "uraemic lung" "uraemic pneumonitis" "uraemic pneumonia" "pulmonary changes in uraemia" "pulmonary manifestations of azotaemia" (26 118 120 123 126 140 259 262) and of myocardial failure in nephritis" "heart failure in acute nephritis" "heart failure and pulmonary oedema" "pulmonary congestion in uraemia" (54 60 196 253) Other designations are "pulmonary changes in hypertension" "pulmonary changes in nephritis" (124 205)

Eventually the terms "fluid lung" (13 16 17) "fluid retention lung" (21) and "uraemic fluid lung" (244) were introduced

In this survey the neutral designation "uraemic pulmonary oedema" will be used.

## Literary survey on the problems of uraemic pulmonary oedema

The *clinical picture* of clinically manifest severe uraemic pulmonary oedema resembles that of pulmonary oedema of other origin (59, 91 and others). The auscultatory findings, on the other hand, may be minimal even in cases of relatively extensive radiographic changes (13, 32, 63). Pleural effusion is present in small or moderate amounts in about 50% of the cases (39). Facial oedema is common, especially in cases of acute renal insufficiency whereas oedema of the legs is rare (20). The patients exhibit no or only slight dyspnoea and no cyanosis; tachypnoea occurs but its correlation with the degree of pulmonary oedema is relatively poor in patients with uraemic pulmonary oedema, alveolar ventilation is reduced and diffusely distributed among all parts of the lung, and vital capacity declines (92). Fever is not a clinical feature (82). Tachycardia is common (11, 125). Moderate anaemia and a slight decrease of serum-proteins are usual features. In most cases the patients have hypertension of varying degrees, but pulmonary changes can also occur in patients who have a low blood pressure or are in shock (3, 11, 18, 20, 21, 106). The hypertension is usually reduced or

disappears after the pulmonary oedema has been brought under control by dehydration. Occasionally however the blood pressure will rise in response to removal of the oedema (3). Many overhydrated patients, with or without oedema of the lungs, show signs of cerebral oedema, such as restlessness, personality changes, mental confusion, and convulsions (20, 21). Ascites does not seem to be a common feature.

Uraemic pulmonary oedema in patients can be complicated by bronchitis and stagnation of secretion, atelectasis, bronchopneumonia, haemorrhages, etc., and, notably in nephrosclerotics and chronic glomerulonephritis, also by congestion as a result of cardiac insufficiency (11, 18, 20, 21, 81, 92, 102, 103, 163, 213).

The *microscopical picture* of uraemic pulmonary oedema at autopsy has been described by several authors (31, 32, 63, 126 and others), who observed *hyperaemia in the lung capillaries*, thickening of the alveolar septa, fibrinous or albuminous *alveolitis* with partial hyalinization of the intra-alveolar exudate, and hyaline membranes clothing the alveolar walls. Small haemorrhages intra-alveolarly and interal-

ment, hypertension, dyspnoea and raised venous pressure.

X ray in the diagnosis of pulmonary oedema in renal disease was first used by Ehrlich & McIntosh (1932) but Zdansky (1933) and Roubier & Plachu (1934) were among the first to describe the more characteristic features. Later numerous authors have reported results and discussed the X ray findings and their pathogenesis (6 10 11 16 20 21 26 31 32 38, 39 43 54 60 63 64 65 91 103 105 118, 120 122 123 125 126 131 132 136 140 144 145 157 160 166 171 193 194 197 198 205 216 216 222 227 230 251 259 261 262)

The radiographic changes characteristic of pulmonary oedema in patients with renal disease have been called by many names, because of the varying views held on their pathogenesis. Some authors have used unspecific designations such as pulmonary oedema (122 125) acute pulmonary oedema (261) "chronic pulmonary oedema (194 251) Among the most usual designations are "pul-

monary oedema in renal insufficiency "pulmonary oedema in uraemia "uraemic oedema of the lungs and uraemic pulmonary oedema (38 60 63 106 145 222) Some talk about pulmonary oedema of renal origin "nephrogenic pulmonary oedema" (131 144 160) others about "uraemic lung "uraemic pneumonitis "uraemic pneumonia" pulmonary changes in uraemia "pulmonary manifestations of azotaemia" (26 118 120 123 126 140 259 262) and of myocardial failure in nephritis "heart failure in acute nephritis" "heart failure and pulmonary oedema" pulmonary congestion in uraemia (64 60 196 253) Other designations are "pulmonary changes in hypertension" pulmonary changes in nephritis (124 205)

Eventually the terms fluid lung" (13 16 17) fluid retention lung" (21) and uraemic fluid lung (244) were introduced.

In this survey the neutral designation uraemic pulmonary oedema" will be used.

nary oedema of hilar type occurs only in association with some degree of uraemia Borgström et al (1900) also postulated that the hilar type signifies renal disease. Several authors have however described cases of cardiac insufficiency with pulmonary changes of hilar type (60 64 10 132, 194) Gould & Torrance (1935) found that the hilar type was most frequent in patients with renal diseases but that the borderlines between the said types of pulmonary changes were not distinct.

The radiographic picture is often complicated, since in patients with renal disease several pathological processes occur at the same time, such as renal and cardiac insufficiency bronchopneumonia haemorrhages and atelectasis. The heart is often enlarged and the diaphragm raised because of abdominal distension. Under such conditions the diagnosis and the grading of pulmonary oedema are difficult (20 21 39 (22 108) In rare cases, X ray can be normal in acute pulmonary oedema with protein-rich transudate even in patients who die from suffocation due to profuse froth in (39)

**Pathogenesis.** According to Altschule (1954) the fundamental disorder in pulmonary oedema seems to be a rate of transudation from the pulmonary capillaries that exceeds the reabsorptive capacity of the pulmonary lymphatics. The following factors contribute to this imbalance 1) Increased transudation caused by a) elevated capillary pressure in the lungs, b) increased filtering area in the lungs, c) large blood

flow in the lungs, d) lowered plasma protein level e) increased capillary permeability and f) bronchospasm 2) Decreased reabsorption owing to impaired lymphatic function 3) Increased total extracellular fluid volume According to Mackeen (1960) no single mechanism is responsible for all forms of pulmonary oedema

Uraemic pulmonary oedema occurs in a variety of renal diseases, such as acute and chronic glomerulonephritis, tubular necrosis, nephrosclerosis, and polycystic kidneys the incidence in the various renal diseases has not been made clear Alwall (1960 and 1963) found that the incidence of "fluid lung" was about equally high in anuric-oliguric patients with tubular necrosis (39 % of 396 patients) glomerulonephritis (40 % of 115 patients) pyelonephritis (46 % of 24 patients) and postrenal obstruction (50 % of 52 patients) but slightly higher in patients with nephrosclerosis (70 % of 10 patients) The incidence was influenced by the duration and degree of severity of the disease (milder cases are less exposed to the risk of overhydration) the presence of overhydration at admission (when most cases of "fluid lung" occurred) a tendency to shock (more fluid had to be given in such cases) and the presence of pneumonia and other conditions which rendered a correct X ray diagnosis difficult The question whether the pathogenesis of pulmonary oedema differs in different renal diseases has not been settled.

Some of the factors that are considered to play a part in causing uraemic pulmonary oedema are



ally (59 118) bronchiolitis (75 255) and focal atelectasis with emphysema (126) have also been observed. Some have noted a profuse amount of protein in the alveolar exudate (e.g. 126) but a low concentration of protein has also been reported (118).

The microscopical changes are not pathognomonic. Similar changes are seen in radiation pneumonia virus pneumonia rheumatic pneumonia respiration atelectasis in the newborn lupus erythematosus periarthritis nodosa and after inhalation of toxic substances (e.g. 26 31 32 106). Bronchiolitis can occur in association with infection or inhalation of chemical substances (75). Bass et al. assumed that in the above-mentioned conditions there would be disturbances in permeability.

The radiographic abnormalities described as typical of uraemic pulmonary oedema are bilateral central diffuse changes, most marked in the hilar region surrounded by free peripheral zones of normal parenchyma (222 261). Unilateral changes may occur (103 125 194 and others) which may be caused by bed rest in the side position (21). The changes can alter their appearance or disappear within a short time (6 16 17 18 103 171 197 251). Pleural effusion is invariably present but usually found in small amounts at examination in the side-position (16 20 38). There is no correlation between the amount of fluid in the pleura and the degree of pulmonary oedema assessed radiographically (39). Oedema of the lungs can be found

both in patients with normal sized hearts and in patients with enlarged hearts (261). Out of 200 patients (also described in Alwall's et al. series) with radiographic evidence of uraemic pulmonary oedema half had enlarged hearts as a result of dilatation hypertrophy or pericarditis (39). In cases of massive pulmonary oedema the changes extend peripherally and the free zones disappear after dehydration the changes disappear first at the periphery (20 21).

The "batwing shadow" (125) seen in the radiograph which has often been described as characteristic, has been given several explanations. Insufficient flow of lymph in the hilar region (71) stagnation of blood in the hilar region (61) reduced blood flow peripherally (212) the bronchial vessels do not extend peripherally in the lung (38) the function of the central parts of the lung is different from that of the cortex (122) or that the whole lung undergoes a change but that the thick layers of the central parts are more clearly visible in the radiograph (20 21).

The question whether it is possible to distinguish radiographically a lung oedema of cardiac origin from a lung oedema of renal origin seems to be left open. Both Zdansky (1933) and Goodrich (1948) considered that pulmonary oedema with hilar extension in the radiograph would be acute pulmonary oedema and with extension basally and peripherally it would be chronic pulmonary oedema notably cardiac insufficiency. Rendich et al. (1941) considered that typical pulmo-

extravascular fluid volume was increased in uraemic patients without clinically demonstrated oedema. Allschulte (1954) stated that the increase in extracellular fluid which often occurs in, for instance, acute nephritis results in a tendency to pulmonary oedema: the explanation would be that, except for the skin, the lung is the organ that is richest in collagen tissue, in which extracellular fluid accumulates.

*Cardiac failure:* Reports found in the older literature concern mainly patients in the terminal stage of chronic renal disease, who had marked hypertension, hypertrophy of the heart, and severe electrocardiographic changes. This led many authors to attach special significance to cardiac insufficiency as the cause of pulmonary oedema in renal disease (e.g. 32, 61 132, 145 180 215 221). Anthonisen considered that many facts favour the theory that pulmonary oedema in chronic renal disease is a result of cardiac failure, in that arteriosclerotic or hypertensive heart-disease, uraemic pericarditis, electrolyte disturbances, and, perhaps, toxic substances in uraemia, as well as anaemia have an inhibitory action on the heart: while, at the same time, the demands on the heart are increased by fever, anoxia, muscular tension, twitchings, and anaemia. De Bass et al. (1956) believed that the main cause would be electrolyte fluid retention resulting from renal insufficiency, hypervolaemia, and secondary heart failure.

Other authors claim however that pulmonary oedema occurs in patients with a normal-sized heart, a normal electrocardiogram, normal clinical findings and a normal heart at autopsy: digitalis has no effect (3 8 10 11 26, 54 60 91 142 and others). Various types of radiographic lung-changes occur in primary cardiac insufficiency and in renal insufficiency (38 103 105). As regards the fact that an increased heart size in pulmonary oedema returns to normal when the oedema is brought under control (241) Allschulte (1954) made the following comment: The enlargement of the heart need not signify insufficiency but can be secondary to fluid retention.

Cardiac catheterizations have been reported in only a few cases with pulmonary oedema predominantly in patients with acute glomerulonephritis. Davies (1951) noted a normal cardiac output. Eichna et al. (1951) observed that the cardiac output was normal but the pressure in the pulmonary artery and right atrium was raised. Morita (1957) reported raised cardiac output, raised pressure in the pulmonary artery, the right atrium and the lung capillaries, and a reduced arteriovenous oxygen difference. De Fazio (1959) noted a markedly increased cardiac output, raised pressure in the pulmonary artery and the lung capillaries, but only slightly raised pressure in the right atrium. Some of our overhydrated uraemic patients with fluid retention lung and arterial hypertension showed virtually normal pressure (catheterizations performed by Inge

*Uraemia* Oedema of the lung in association with renal disease is predominantly reported in patients with uraemia. In rare cases, however pulmonary oedema of this type occurs in patients with slight uraemia (21 216) and in patients with suspected renal damage without uraemia. Cases of the latter type have been described in patients with malignant hypertension (65 115 124 125 132) and acute glomerulonephritis (103 255) during pregnancy (132) and in newly operated patients (136)

Toxic substances of various kinds are considered to cause an increase in capillary permeability in uraemia (13 38 63 103 105 118 122 262 and others) Uraemic pulmonary oedema can disappear if the uraemia is reduced by dialysis, even if the body weight increases (120 182)

There is no correlation between the degree of uraemia and the extent of the pulmonary changes (21 63 215) At X ray examination of a large number of patients with severe uraemia only a few were found to have changes of pulmonary-oedema type (215) Oedema of the lungs in uraemic patients occurs among those who fall ill with acute anuria as well as in those who have had uraemia for many years (20 21) If a uraemic patient with pulmonary oedema is dehydrated the pulmonary changes disappear even though the uraemia continues to increase (13 18 20 21) Nor has any correlation been demonstrated between the concentration of urea, creatinine, uric acid acidosis etc. and the presence of pulmonary oedema.

*Fluid retention* Exogenous overhydration resulting from, for instance parenteral infusions or endogenous overhydration due to inadequate elimination of the water produced in catabolism can cause pulmonary oedema in renal insufficiency (6 10 13, 16, 17 18 19 20 21 103 194 216 and others) Dangerous fluid therapy without weight-control in cases of acute nephritis (to make diuresis recommence or increase) can lead to fatal oedema (10) The radiographic changes consistent with pulmonary oedema can disappear in response to dehydration (6 10 11 13 16 17 20 21 160 261) The pulmonary changes can be made to disappear in a few hours by diarrhoea induced with sodium sulphate or sorbitol (6, 10 11 13 16 18, 20 21) or by ultrafiltration of the blood in vivo (6 10 11 12 13 16 17 19 20 21)

Alwall (1952) and Alwall Lunderquist & Olsson (1953) referring to the above mentioned clinical results (Alwall & Olsson) and experimental results in animals (Alwall & Lunderquist) proposed that the terms "uraemic lung" etc. should be replaced by "fluid lung" as this designation would give information about the pathogenesis and point to the adequate treatment. Alwall later (1963) used the term "fluid retention lung" Few studies on the body's fluid spaces in pulmonary oedema have been reported. A markedly increased extravascular alcohol space in the lung of a patient with pulmonary oedema has been observed (122)

Nickel et al. (1953) noted that the

extravascular fluid volume was increased in uraemic patients without clinically demonstrated oedema. Allschule (1954) stated that the increase in extracellular fluid which often occurs in, for instance, acute nephritis results in a tendency to pulmonary oedema the explanation would be that, except for the skin, the lung is the organ that is richest in collagen tissue, in which extracellular fluid accumulates.

**Cardiac failure** Reports found in the older literature concern mainly patients in the terminal stage of chronic renal disease, who had marked hypertension, hypertrophy of the heart, and severe electrocardiographic changes. This led many authors to attach special significance to cardiac insufficiency as the cause of pulmonary oedema in renal disease (e.g. 32 64 132 145 160 216 221) Anthonisen considered that many facts favour the theory that pulmonary oedema in chronic renal disease is a result of cardiac failure, in that arteriosclerotic or hypertensive heart-disease, uraemic pericarditis, electrolyte disturbances, and, perhaps, toxic substances in uraemia, as well as anaemia have an inhibitory action on the heart, while, at the same time, the demands on the heart are increased by fever anoxia, muscular tension, twitchings, and anaemia. De Bass et al. (1956) believed that the main cause would be electrolyte-fluid retention resulting from renal insufficiency hypervolaemia, and secondary heart failure.

Other authors claim however that pulmonary oedema occurs in patients with a normal-sized heart, a normal electrocardiogram normal clinical findings, and a normal heart at autopsy digitalis has no effect (3 6, 10 11 26 54 80 91 142 and others) Various types of radiographic lung-changes occur in primary cardiac insufficiency and in renal insufficiency (58, 103, 105) As regards the fact that an increased heart size in pulmonary oedema returns to normal when the oedema is brought under control (241) Allschule (1954) made the following comment The enlargement of the heart need not signify insufficiency but can be secondary to fluid retention.

Cardiac catheterizations have been reported in only a few cases with pulmonary oedema, predominantly in patients with acute glomerulonephritis. Davies (1951) noted a normal cardiac output. Elchma et al. (1954) observed that the cardiac output was normal but the pressure in the pulmonary artery and right atrium was raised. Morita (1957) reported raised cardiac output, raised pressure in the pulmonary artery the right atrium and the lung capillaries and a reduced arteriovenous oxygen difference. De Fazio (1950) noted a markedly increased cardiac output, raised pressure in the pulmonary artery and the lung capillaries, but only slightly raised pressure in the right atrium. Some of our overhydrated uraemic patients with fluid retention lung and arterial hypertension showed virtually normal pressure (catheterizations performed by Inge

Edler M D) in the right atrium as well as in the pulmonary artery and capillaries (Alwall 1963) Anthonisen (1980) found a normal cardiac output in uraemia without oedema

Kassirer & Schwartz (1961) stressed the difference between pulmonary oedema resulting from primary heart failure and pulmonary oedema occurring in salt and water retention in acute glomerulonephritis, acute renal failure, anaemia, steroid overdosage, and beriberi. In contrast to heart failure, the last mentioned conditions are attended with normal or increased cardiac output, small arterio-venous oxygen differences and normal or reduced total red cell volume and digitalis has no beneficial effect on pulmonary oedema of the latter cause. The authors therefore considered it doubtful whether one can speak about heart failure in these conditions.

**Hypertension** This is a common phenomenon in uraemic pulmonary oedema (3, 20, 23, 196 and others). The aetiological role of hypertension in pulmonary oedema remains to be clarified, but whatever mechanisms are ultimately found it is clinically evident that hypertension is important in the genesis of pulmonary oedema (3). Eichna et al (1954) found a low pressure in the heart, normal blood volume, and a seemingly normal heart in a patient with pulmonary oedema. They considered that these findings could be attributable to a redistribution of the blood volume to the lungs from other vascular areas. A similar assumption was made in connection

with pulmonary oedema of renal type in a hypertensive patient with pheochromocytoma (105). In animals, pulmonary oedema with a similar mechanism can be induced by injection of adrenaline. The following arguments have been presented against hypertension as the cause of circulatory congestion in acute glomerulonephritis: 1) The blood pressure seldom rises above 180/100 mm Hg in these cases. 2) There is no correlation between hypertension and circulatory congestion. 3) Some patients have circulatory congestion without hypertension (142, 196).

**Some other pathogenic factors** Hypoxia has been thought to play a part in causing increased permeability in the lung capillaries and pulmonary oedema in renal disease (32, 105). Marked stagnation of secretion, atelectasis and bronchopneumonia are common in uraemic patients (60, 85, 92) with diminished  $pO_2$  as a result (81). Complicating infection in the lungs and tachypnoea have been considered as causal factors in pulmonary oedema associated with renal disease (60).

Hypoxia does not seem to be the whole explanation of pulmonary oedema since a vicious circle would rapidly be set up and thus, every pulmonary oedema would always become irreversible (103). Normal oxygen saturation occurs even in uraemic patients with slight pulmonary oedema (60) and many patients with uraemia have normal  $pO_2$  (21). When uraemic patients have hypoxia it is in many cases

due to causes other than pulmonary oedema (81)

Anaemia has been mentioned as a factor concerned in the causation of uraemic pulmonary oedema (64) Patients with chronic anaemia of various origin have a high cardiac output and are likely to develop pulmonary oedema after infusions (233) The mechanism has not been made clear

Cerebral damage has been suggested as a causal agent in uraemic pulmonary oedema (103 105 122) The cere-

bral factor would act via a change in the vagal tone (51) or respiration (3) However cerebral oedema occurs independently of pulmonary oedema (20)

Changes in the amount of plasma protein are relatively slight in most renal patients with pulmonary oedema. The lack of correlation between pulmonary oedema and hypoproteinemia is evident from the fact that pulmonary oedema seldom occurs in nephrosis (181 238)

## Literary survey of experimental uraemic pulmonary oedema

Experimental pulmonary oedema was first induced by Cohnheim & Lichtheim in 1850—1860. Several surveys of the literature on experimentally produced pulmonary oedema have been published (e.g. 108, 202, 246).

Viascher et al. (1956) summarized the various experimental methods of producing pulmonary oedema as follows: 1) Primary haemodynamic alterations (operations on the heart and the large vessels, hypervolaemia, arterial hypotension, embolism); 2) Alterations of the central nervous system (brain lesions, compression or stimulation); 3) Alterations of the peripheral nervous system (vagotomy, faradic stimulation of lung root); 4) Alterations of the respiratory system (airway obstruction, hypoxia, drowning, intratracheal fluids etc.); 5) Pharmacological effects (muscarine, histamine, adrenaline, lung irritant gases, bile salts, barbiturates, and others); 6) Miscellaneous (hyperthermia, sensitively phenomena, mechanical irritation of the bronchus).

The tendency to develop pulmonary oedema varies from one animal species to another. Welch as early as 1878 observed that rabbits were more apt to develop lung oedema than were dogs. The pathogenesis of experimentally produced pulmonary oedema is not always the same. The history of the

methods used in this work will be surveyed at the beginning of each chapter.

Alwall (1952), Alwall & Lunderquist (1953), Lunderquist (1953) and Alwall (1954, 1962) in experiments with rabbits whose ureters had been ligated, tried to induce slowly developing pulmonary oedema which could give a radiographic picture similar to the clinical picture of uraemic pulmonary oedema. Overhydration was not sufficient. The toxic effect of Narkotal (Alwall et al. 1949) was therefore utilized as a basis. Short anaesthesia induced with Narkotal and overhydration gave a high frequency of radiographic pulmonary changes (Narkotal uraemia fluid lung) within a few days in anuric rabbits. A detailed account of these experimental series will be found in Chapter 10.

Borgström, Ising, Linder & Lunderquist (1960) aimed at obtaining a radiographic picture which they regarded as typical of uraemic pulmonary oedema. The uraemic pulmonary oedema is characterized by central changes, surrounded by a free peripheral zone of normal lung parenchyma 2 to 4 cm in thickness and slightly broader at the lobe limits. A similar radiographic picture was obtained in dogs, when gull

salts were injected into the bronchial artery at catheterisation. Injection of gall salts into the pulmonary artery or clamping of the aorta produced radiographic pulmonary oedema which, however did not show the above-stated typical picture. After injection of gall salts into the bronchial artery no enlargement of the heart, insignificant pleural effusion, insignificant oedema in the alveoli or air passages, and normal pressure in the lung capillaries were noted, but hypertension in the pulmonary artery and periarterial oedema were obtained. The pulmonary oedema induced by injection of gall salts into the bronchial artery showed such a good radiographical, micro-

scopical, and clinical resemblance to the radiographic uraemic pulmonary oedema described in man that the authors formed the hypothesis that a change in the permeability of the bronchial capillaries may be the prime aetiological factor in the production of uraemic pulmonary oedema. Consequently a reduction in the osmotic pressure of the blood produced by overhydration and/or a complicating left heart failure would be enhancing factors.

Changes in the serum-electrolyte concentration in animals with acute anuria have been described by for instance Harrison et al. (1932)



## Technique

*Surveys of methods* for measuring the degree of experimental pulmonary oedema have been published by Poulsen (1934) and Vischer Haddy & Stephens (1956).

According to Vischer et al. 9 methods are available for evaluation of the oedema state: 1) Auscultation of the chest 2) Observation and collection of frothy fluid in the larger air ways during life 3) X-ray density studies during life 4) Gross observation at autopsy 5) Microscopical observation of autopsy material 6) Measurements of lung weight to body or other organ weight ratios at autopsy 7) Chemical analysis of extravascular extracellular fluid space in the lung at autopsy 8) Changes in electrical resistance of the lung parenchyma 9) Weight changes in the isolated perfused organ. Poulsen mentioned some of these methods and added 10) The proportion of the lung volume to body weight 11) Dry matter determination of the lung. He also mentioned that 12) Drinker (1947) measured the amount of lymph flowing from the lung and used this as a measure of the amount of fluid penetrating through the walls of the capillaries and passing into the lymph vessels.

In the present studies which were made on rabbits, the following 10 methods were used in determining the degree of pulmonary oedema: 1) Assessment of the appearance and behaviour of the animals 2) Respiratory rate 3) Electrocardiographic examination 4) X-ray of the lungs 5) Gross examination

of the lungs 6) Estimation of froth in the air ways post mortem 7) Measurements of lung weight, absolute and in relation to body weight 8) Determination of the water content of the lung 9) Microscopical examination of the lungs and 10) Lung volume, in a few experiments.

Rabbits weighing 2.20–2.40 kg. were used; all were of the same breed, but with coats of different colours, and of both sexes. Each animal was placed alone in a cage, with free access to water and was given food in the form of pellets. The pellets contained corn, wheat bran, oil concentrates, fish meal, meat meal, Alfalfa leaf meal, skim milk powder, bone meal and calcium and vitamins (A, B, D). On this diet the rabbits gained satisfactorily in weight. By daily checking of the body weight and by the clinical picture the well-being of each animal was ascertained. 90% of the rabbits were kept in their cages for at least 1 (1–30) days before the beginning of the experiments.

*The appearance and behaviour of the animals.* The power of reaction and the wakefulness of the animals could be assessed by, for instance, spontaneous movements, nose movements, the position of the head and the reaction to different stimuli such as touching the nose, blowing into the outer ear.

and the corneal reflex. The presence of cerebral disorders could be established by unsteady gait, nystagmus, exophthalmos, restlessness, twitchings, convulsions, and loss of reflexes. Posture respiration, and the size of the orifices of the nose gave information about respiratory difficulties. Anaemia, cyanosis, and the filling of the vessels were ascertained by studying the conjunctivae and the mucous membranes of the nose and mouth. When they had been taken ill, the animals lay flat on their abdomens with paws outstretched, or remained lying in the side position without trying to rise. Stethoscope examination was not made. This method was by Lunde & Sarnoff (1970) considered to be unsatisfactory.

*Respiratory rate* was measured before and after procedures aimed at inducing pulmonary oedema (infusions, etc.) possibly in the sitting position.

*The heart-rate and electrocardiograms* were recorded with the Mingograf (Elema Schöander Stockholm). Changes in the normal electrocardiogram of rabbits have been described by, for instance, Levine (1942) and Lepeschkin (1967). Levine found that the heart-rate in rabbits at rest was 225 (174–283) beats per minute and that an inverted T wave was present in only 1 of 23 cases in lead I in naturally sitting rabbits.

Electrocardiograms were recorded before ligation of the ureters as well as before and after procedures to induce pulmonary oedema. Some ten heart beats were registered in one lead. Trembling and movements of the animals and respiration could produce

disturbances. It was sometimes difficult to find optimal positions for the electrodes.

*Chest radiographs* were taken in the anteroposterior view before ligation of the ureters, before and after procedures to induce pulmonary oedema, and before the animals were bled to death for the purpose of demonstrating pulmonary changes. A few rabbits which died spontaneously were examined immediately after death. Examinations in the lateral position for the presence of pleural fluid *in vivo* were made in a few series.

Chest radiographs in experimentally produced pulmonary oedema have been published by Lunde (1948) Alwall, Lunderquist *et al.* (1952–1963) (also in the lateral position) and Calkins (1960). As an argument against the use of chest X-ray Vischer *et al.* (246) referring to Westermark (1948) stated that distended blood vessels may be visible long shadows, ordinarily interpreted as evidence of pulmonary oedema, and that X-ray evidence of nasal oedema occurs only when the oedema has approached lethal degree.

Our laboratory X-ray apparatus, which is fitted with an optical centering device, allows exposures of 0.2 second at 52 kV and 570 mA. The rabbit was fixed on table and enclosed in iron plating, with a window cut out over the chest, and was thus held in the proper position. The film was taken with the rabbit in the hanging position (Fig. 1) the tube-film distance was 100 cm. The technique has been described by Alwall & Lunderquist (1952) and will be described in detail by Lunderquist in 1964 (in the press).

Dr Anders Lunderquist at the X-ray Department I Lund (Head Olle Ol-

## Technique

Surveys of methods for measuring the degree of experimental pulmonary oedema have been published by Poulsen (1954) and Vlascher Haddy & Stephens (1956).

According to Vlascher et al., 9 methods are available for evaluation of the oedema state: 1) Auscultation of the chest 2) Observation and collection of frothy fluid in the larger air ways during life 3) X ray density studies during life 4) Gross observation at autopsy 5) Microscopical observation of autopsy material 6) Measurements of lung weight to body or other organ weight ratios at autopsy 7) Chemical analysis of extravascular extracellular fluid space in the lung at autopsy 8) Changes in electrical resistance of the lung parenchyma 9) Weight changes in the isolated perfused organ. Poulsen mentioned some of these methods and added: 10) The proportion of the lung volume to body weight 11) Dry matter determination of the lung. He also mentioned that Drinker (1947) measured the amount of lymph flowing from the lung and used this as a measure of the amount of fluid penetrating through the walls of the capillaries and passing into the lymph vessels.

In the present studies, which were made on rabbits, the following 10 methods were used in determining the degree of pulmonary oedema: 1) Assessment of the appearance and behaviour of the animals 2) Respiratory rate 3) Electrocardiographic examination 4) X ray of the lungs 5) Gross examina-

tion of the lungs 6) Estimation of froth in the air ways post mortem 7) Measurements of lung weight, absolute and in relation to body weight 8) Determination of the water content of the lung 9) Microscopical examination of the lungs and 10) Lung volume, in a few experiments.

Rabbits weighing 2.20—2.49 kg. were used; all were of the same breed, but with coats of different colours, and of both sexes. Each animal was placed alone in a cage, with free access to water and was given food in the form of pellets. The pellets contained corn, wheat bran, oil concentrates, fish meal, meat meal, Alfalfa leaf meal, skim milk powder, bone meal and calcium and vitamins (A, B<sub>2</sub>, D<sub>3</sub>). On this diet the rabbits gained satisfactorily in weight. By daily checking of the body weight and by the clinical picture the well being of each animal was ascertained. 90% of the rabbits were kept in their cages for at least 1 (1—30) days before the beginning of the experiments.

*The appearance and behaviour of the animals.* The power of reaction and the wakefulness of the animals could be assessed by, for instance, spontaneous movements, nose movements, the position of the head and the reaction to different stimuli such as touching the nose, blowing into the outer ear.

Bloodletting was chosen in our experiments after comparison to, for instance, electrocution and intravenous injection of air or 'arkotal'. It does not seem advisable to use methods that could cause or aggravate pulmonary oedema, haemorrhages, hyperaemia, or atelectasis. On the other hand, in my experience bloodletting can be made more or less complete which may influence the atopsy findings (the blood supply in the vessels and the weight of the lungs). The preparations are also fairly time-consuming and many of our animals died spontaneously before the planned bloodletting could be started.

The following technique was used at autopsy. When breathing had ceased the trachea was ligated tightly. The chest was opened with scissors by cutting off the costal process and some of the lower ribs. The heart was in most cases beating at the beginning of the autopsy. The fluid in the pericardial sac was sucked out by means of a small pipette. The entire anterior portion of the chest was then cut open and removed together with the sternum and the anterior parts of the ribs. The root of the lung was ligated bilaterally including vessels and bronchi. The pleural fluid was sucked out of the pleural cavities. The heart-lung preparation was freed caudally and lifted out of the chest. The trachea was cut open and examined for the presence of frothy fluid. The trachea, the oesophagus, and other attachments were then severed. Each lung was cut off separately from the heart-lung preparation and placed in an open beaker of thin aluminium foil. The thread around the root of the lung together with vessels and bronchial portions outside the lung were cut away. The lungs were examined macroscopically and weighed. The water content of the lung was estimated by exsiccation of the cut surface through the lung and by judging how easily fluid could be expressed, and was coded as +, ++ and +++ The left lung was used for determination of the water content. From the most markedly changed parts of

the right lung, pieces were taken for microscopical examination. These were usually of hazelnut size, on a few occasions consisting of whole lobes. If no gross pulmonary changes were seen, and when microscopical examination was considered warranted, pieces were taken from the basal and hilar parts of the right lower lobe in which, empirically, the incidence of pulmonary changes is highest. The pieces were fixed in 10% formalin. The amount of peritoneal fluid was measured by pouring the abdominal contents into a dish after cutting open the abdomen and, after a little while, removing the viscera so that only the peritoneal fluid remained in the dish.

Gross observation at autopsy comprised the size of the lungs, the moisture of the surfaces, and the appearance of the lung surface, the latter being the most important factor. The lung surface showed the following features in different conditions.

In normal lungs the surface was smooth and pink.

Areas of atelectasis were in most cases seen in both lower lobes, sometimes in the smaller lobes and occasionally locally at the lung borders. The lungs were small in cases of extensive atelectasis. The colour was bluish-purple or brown, occasionally purple with white spots.

Areas of pneumonia were as a rule reddish brown and clearly defined.

Hyperaemia was seen in animals which had died spontaneously or after incomplete bloodletting. The lung surface was dark red and blood ran profusely from the section surface.

Haemorrhages were manifested by small red dots in a few cases, spots, or rarely large areas of bleeding. In

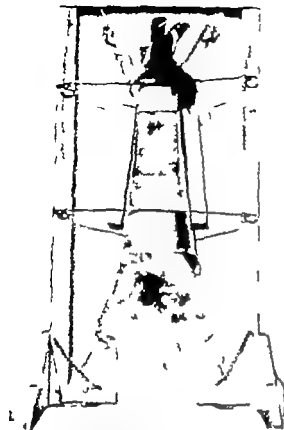


Figure 1 The position of the rabbit fixed to the table during X ray examination. The animal's back is enclosed in a metal shield provided with a window

son M D) examined the films without knowing from which series they were taken but being informed of the amount of pleural effusion and ascites at autopsy. Radiographic changes consistent with pulmonary oedema and changes of other type were recorded. The extent to which the changes were conditioned by factors other than pulmonary oedema was not assessed, however. The heart size was estimated and graded as "0 + ++ and +++".

The following typical set of pictures will serve as an example

Fig 3 shows the chest film of a normal rabbit

Figs 4 5 6 and 7 show different degrees of pulmonary oedema coded as "+ ++ and +++"

Fig 8 shows pneumonia in the upper lobes of a vagotomized rabbit

Fig 9 shows atelectasis in profuse pleural effusion

Fig 10 shows large pulmonary haemorrhages

Fig 11 shows cardiac enlargement +++ without any pulmonary change.

#### Gross observation at autopsy

Durlacher et al. (1950) examined the lung weight after seven different methods of killing the rabbits (bloodletting, ether anaesthesia, intravenous injection of air, electrocution, rabbit punch,  $MgSO_4$  intravenously and Nembutal intravenously). Only a slight difference in the weight of the lungs, irrespective of manner of death, was noted if the rabbits were examined immediately after death. In rabbits examined after lying dead for 3 hours the lung weight did not rise in those killed by bloodletting but increased markedly after all the other methods, and most markedly after death from acute barbiturate poisoning.

Injection of 275 mg of Narkotal intravenously gave in our experiments hyperaemia in 4 rabbits examined 10 minutes after death, and massive pulmonary oedema in 4 rabbits examined about 25—30 minutes after death. Chest X ray examinations made about 5 minutes post mortem (2 animals had then frothy fluid in the nose) showed no abnormalities. Pulmonary oedema can possibly arise very quickly after death, but the possibility that pulmonary oedema can be present even if the radiograph is normal, should always be considered.

Bloodletting was chosen in our experiments after comparison to for instance, electrocution and intravenous injection of air or  $\text{N}_2$  (Klotz). It does not seem advisable to use methods that could cause or aggravate pulmonary oedema, haemorrhages, hyperaemia, or atelectasis. On the other hand, in my experience bloodletting can be made more or less complete which may influence the autopsy findings (the blood supply in the esaei and the weight of the lungs) the preparations are also fairly time-consuming and many of our animals died spontaneously before the planned bloodletting could be started.

The following technique was used at autopsy. When breathing had ceased the trachea was ligated lightly. The chest was opened with scissors by cutting off the ensaei rim process and some of the lower ribs. The heart was in most cases beating at the beginning of the autopsy. The fluid in the pericardial sac was sucked out by means of a small pipette. The entire anterior portion of the chest was then cut open and removed together with the sternum and the anterior parts of the ribs. The root of the lung was ligated bilaterally including vessels and bronchi. The pleural fluid was sucked out of the pleural cavities. The heart-lung preparation was freed caudally and lifted out of the chest. The trachea and chest was cut open and examined for the presence of frothy fluid. The trachea, the oesophagus, and other attachments were then severed. Each lung was cut off separately from the heart-lung preparation and placed in an open beaker of thin aluminium foil. The thread around the root of the lung together with vessels and bronchial portions outside the lung were cut away. The lungs were examined macroscopically and weighed. The water content of the lung was estimated by examination of the cut surface through the lung and by judging how easily fluid could be expressed, and was coded as +, ++ and ++++. The left lung was used for determination of the water content. From the most markedly changed parts of

the right lung, pieces were taken for microscopical examination these were usually of hazelnut size, on a few occasions consisting of whole lobes. If no gross pulmonary changes were seen, and when microscopical examination was considered warranted, pieces were taken from the basal and hilar parts of the right lower lobe in which, empirically the incidence of pulmonary changes is highest. The pieces were fixed in 10% formalin. The amount of peritoneal fluid was measured by pouring the abdominal contents into a dish after cutting open the abdomen and, after a little while, removing the viscera so that only the peritoneal fluid remained in the dish.

Gross observation at autopsy comprised the size of the lungs, the moisture of the surfaces, and the appearance of the lung surface, the latter being the most important factor. The lung surface showed the following features in different conditions.

In normal lungs the surface was smooth and pink.

Areas of atelectasis were in most cases seen in both lower lobes, sometimes in the smaller lobes and occasionally locally at the lung borders. The lungs were small in cases of extensive atelectasis. The colour was bluish purple or brown, occasionally purple with white spots.

Areas of pneumonia were as a rule reddish-brown and clearly defined.

Hyperaemia was seen in animals which had died spontaneously or after incomplete bloodletting. The lung surface was dark red and blood ran profusely from the section surface.

Haemorrhages were manifested by small red dots, in a few cases spots, or rarely large areas of bleeding. In

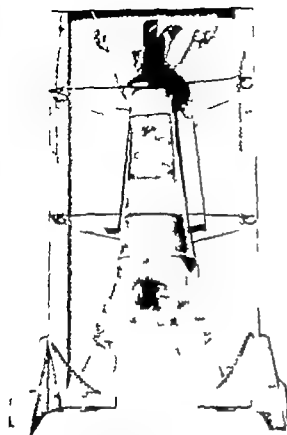


Figure 1 The position of the rabbit fixed to the table during X-ray examination. The animal's back is enclosed in a metal shield provided with a window

son M D) examined the films without knowing from which series they were taken but being informed of the amount of pleural effusion and ascites at autopsy. Radiographic changes consistent with pulmonary oedema, and changes of other type were recorded. The extent to which the changes were conditioned by factors other than pulmonary oedema was not assessed, however. The heart size was estimated and graded as "0 + ++ and +++"

The following typical set of pictures will serve as an example

Fig 3 shows the chest film of a normal rabbit

Figs 4 5 6 and 7 show different degrees of pulmonary oedema coded as "+" "++" and "+++"

Fig 8 shows pneumonia in the upper lobes of a vagotomized rabbit

Fig 9 shows atelectasis in profuse pleural effusion

Fig 10 shows large pulmonary haemorrhages

Fig 11 shows cardiac enlargement "+++" without any pulmonary change.

#### *Gross observation at autopsy*

Durlacher et al. (1950) examined the lung weight after seven different methods of killing the rabbits (bloodletting, ether anaesthesia, intravenous injection of air, electrocution, rabbit punch, MgSO<sub>4</sub> intravenously and Nembutal intravenously). Only a slight difference in the weight of the lungs, irrespective of manner of death, was noted if the rabbits were examined immediately after death. In rabbits examined after lying dead for 3 hours the lung weight did not rise in those killed by bloodletting, but increased markedly after all the other methods, and most markedly after death from acute barbiturate poisoning.

Injection of 275 mg of Narkotal intravenously gave in our experiments hyperaemia in 4 rabbits examined 10 minutes after death, and massive pulmonary oedema in 4 rabbits examined about 25–30 minutes after death. Chest X ray examinations made about 5 minutes post mortem (2 animals had then frothy fluid in the nose) showed no abnormalities. Pulmonary oedema can possibly arise very quickly after death but the possibility that pulmonary oedema can be present even if the radiograph is normal, should always be considered.

Bloodletting was chosen in our experiments after comparison to, for instance, electrocution and intravenous injection of ir or Yarkotal. It does not seem advisable to use methods that could cause or aggravate pulmonary oedema, haemorrhages, hyperaemia, or atelectasis. On the other hand, in my experience bloodletting can be made more or less complete which may influence the autopsy findings (the blood supply in the esei and the weight of the lungs) the preparations are also fairly time-consuming and many of our animals died spontaneously before the planned bloodletting could be started.

The following technique was used at autopsy. When breathing had ceased the trachea was ligated lightly. The chest was opened with scissors by cutting off the ensiform process and some of the lower ribs. The heart was in most cases beating at the beginning of the autopsy. The fluid in the pericardial sac was sucked out by means of a small pipette. The entire anterior portion of the chest was then cut open and removed together with the sternum and the anterior parts of the ribs. The root of the lung was ligated bilaterally including vessels and bronchi. The pleural fluid was sucked out of the pleural cavities. The heart lung preparation was freed caudally and lifted out of the chest. The stretched trachea was cut open and examined for the presence of frothy fluid. The trachea, the oesophagus, and other attachments were then severed. Each lung was cut off separately from the heart lung preparation and placed in an open beaker of thin aluminium foil. The thread around the root of the lung together with vessels and bronchial portions outside the lung were cut away. The lungs were examined macroscopically and weighed. The water content of the lung was estimated by examination of the cut surface through the lung and by judging how much fluid could be expressed, and was coded as + + + and + + +. The left lung was used for determination of the water content. From the most markedly changed parts of

the right lung, pieces were taken for microscopical examination (these were usually of hazelnut size, on a few occasions consisting of whole lobes). If no gross pulmonary changes were seen, and when macroscopical examination was considered warranted, pieces were taken from the basal and hilar parts of the right lower lobe in which, empirically, the incidence of pulmonary changes is highest. The pieces were fixed in 10% formalin. The amount of peritoneal fluid was measured by pouring the abdominal contents into a dish after cutting open the abdomen and, after a little while, removing the viscera so that only the peritoneal fluid remained in the dish.

Gross observation at autopsy comprised the size of the lungs, the moisture of the surfaces, and the appearance of the lung surface, the latter being the most important factor. The lung surface showed the following features in different conditions.

In normal lungs the surface was smooth and pink.

Areas of atelectasis were in most cases seen in both lower lobes, sometimes in the smaller lobes and occasionally locally at the lung borders. The lungs were small in cases of extensive atelectasis. The colour was bluish-purple or brown, occasionally purple with white spots.

Areas of pneumonia were as a rule reddish-brown and clearly defined.

Hyperaemia was seen in animals which had died spontaneously or after incomplete bloodletting. The lung surface was dark red and blood ran profusely from the section surface.

Haemorrhages were manifested by small red dots, in a few cases spots, or rarely large areas of bleeding. In



many cases of long survival time the colour was brownish black.

Markedly developed pulmonary oedema was easily recognisable but in mild forms the assessment was subjective.

Farber (1937) by direct inspection saw pulmonary oedema developing in vagotomized animals. The changes began with petechiae, which fused into larger areas with red discoloration, later found to be consolidated areas. Poulsen (206) described the lungs in excessive oedema as full and heavy with more or less extensive hyperaemia less pronounced pulmonary oedema is not directly visible but must often be suspected in the presence of fairly extensive hyperaemia, which usually precedes or accompanies oedema formation.

In the present study the lung oedema was graded as follows "+++" Mottled lung surface with the surface divided into numerous small diffuse spots of various colour combinations such as reddish pink, dark red to red purple to beige, bluish brown, reddish brown to white. Hilary and basal parts were occasionally wholly dark red or purple

"++ Spotted lung surface, the surfaces of the lower lobes in particular being partly covered with large spots about 1 cm in diameter unevenly and diffusely defined and most often of reddish colour occasionally of brown in rare cases white shades.

+ Dotted lung surface the surface, notably hilary or basally, being studded over with numerous red dots or reddish brown small spots or numerous white dots seen on shining moist surfaces Lungs were rich in

blood and brownish red (transition between hyperaemia and pulmonary oedema) when the variations in colour were asymmetrical and uneven and the changes were distinctly localized bilaterally in the hilar regions. In the vagotomy series, brown changes bilaterally in the upper lobes and parts of the lower lobes were graded as +

*Frothy fluid in the air ways* was noted by site a) the bronchi b) the trachea or c) flowing through the mouth or the orifices of the nose. As far as possible frothy fluid from pulmonary oedema was distinguished from that produced at agony or post mortem by phlegm blood, or aspirated matter Frothy fluid has been graded in other studies (e.g 170)

The *weight of the lungs* has been used by many authors in grading pulmonary changes in experimental animals (17 48 73 114 152, 169 206 218 221 232 237 256)

Durlacher et al. (73) reported a lung weight of  $3.57 \pm 0.21$  and Alwali et al. (21) of 4.0 (3.0—5.2) g per kg body weight in bled rabbits. Luisada (169) quoted a lung weight per kg body weight in rabbits of  $4.55 \pm 0.21$  g in normal animals and  $13.50 \pm 0.35$  g in bled animals which had received adrenaline injections. Courtice (48) found that the lung weight per kg body weight averaged about 4.90 g in normal rabbits, 6.60 g in rabbits with slight pulmonary oedema 6.30 g in those with moderate pulmonary oedema, and 8.10 g in those with severe pulmonary oedema. Stone & Loew (237) noted that in rabbit weighing 2.50 (2.00—3.00) kg and killed by a blow on the head the lung weight was  $10.58 \pm 1.41$  g After a large dose of

adrenaline intravenously the lung-weight rose to  $21.77 \pm 7.10$  g and after phenobarbitone plus adrenaline to  $34.03 \pm 14.49$  g

Two noteworthy arguments have been advanced against the use of the lung weight as a criterion of pulmonary oedema. Durlacher et al. (1930) found that the weight of the lungs increased after death and that with some manners of death in experimental animals it could rise twofold in 3 hours. Poulsen (1934) asserted that increased lung weight could be due not only to pulmonary oedema but also to hyperaemia and pneumonia, and therefore recommended determination of the water content of the lung, since this did not increase in hyperaemia and pneumonia, whereas it did so in pulmonary oedema.

#### *Determination of the water content of the lung*

The water content of the lung has been determined by desiccation (176, 180, 206, 213, 236, and others). Reichsman (213) found that in rabbits the water content in the left lung (the right one was used for microscopical examination) in marked pulmonary oedema averaged 83.2 (80.2–89.5) % whereas in normal rabbits or those with slight pulmonary oedema the water content was 77.1 (73.8–81.2) %. Henderson et al., using the freeze-drying technique, noted an average water content of 79.6 % in the rabbit lung, 85.3 % in blood, and 92.0 % in oedema fluid drying in an oven gave almost identical results. Manery et al. (1939) reported an average water content of 80.2 % in the lung and 87.5 % in the blood. Poulsen (1934) found that in mice the water content of the lung averaged 77.1 % that of the blood 78.5 % and that of oedema fluid 91.9 %. It con-

cluded that it seems evident that the water content of lungs showing a weight increase due exclusively to an increased accumulation of blood does not differ essentially from that of normal lungs. On the other hand, a weight increase caused by oedema fluid will raise considerably the water content of the lungs. He considered that the water content would hence be diagnostic in distinguishing pulmonary oedema from hyperaemia and inflammation.

According to Poulsen, freeze-drying for 48 hours maintains the lung-volume unchanged, whereas heat-drying of organic matter especially that containing blood, causes loss of volume and formation of tough membranes that prevent complete drying.

The method has been criticized by several authors. Wood & Moe (1942) considered that the water content could not be regarded as a reliable criterion of oedema, since the protein concentration in the oedema fluid varies markedly and since the results differ according to the length of drying and the temperature. Vlachar et al. (246) did not consider the method reliable, as the blood-cell/plasma ratio was increased in the blood present in the lung. Reichsman (213) on the other hand, found a close correlation between water content and the amount of pulmonary oedema observed by gross examination.

The following technique was used in the present study: The left lung was placed in a aluminium-foil beaker was cut into small pieces and desiccated under infrared light (Ultra X system Grouart Infrared lamp). The heat radiation from the lamp could be regulated by means of a slide resistor. The desiccation was continued until two weightings at 5-minute intervals gave the same weight. Lung tissue cut in

many cases of long survival time the colour was brownish black.

Markedly developed pulmonary oedema was easily recognisable but in mild forms the assessment was subjective.

Farber (1937) by direct inspection saw pulmonary oedema developing in vagotomized animals. The changes began with petechiae which fused into larger areas with red discoloration later found to be consolidated areas. Poulsen (206) described the lungs in excessive oedema as full and heavy with more or less extensive hyperaemia less pronounced pulmonary oedema is not directly visible but must often be suspected in the presence of fairly extensive hyperaemia which usually precedes or accompanies oedema formation.

In the present study the lung oedema was graded as follows "+++" Mottled lung surface, with the surface divided into numerous small diffuse spots of various colour combinations such as reddish pink, dark red to red, purple to beige, bluish brown reddish brown to white. Hilary and basal parts were occasionally wholly dark red or purple.

++ Spotted lung surface, the surfaces of the lower lobes in particular being partly covered with large spots about 1 cm in diameter unevenly and diffusely defined, and most often of reddish colour occasionally of brown in rare cases white shades.

+ Dotted lung surface, the surface notably hilary or basally being studded over with numerous red dots or reddish brown small spots or numerous white dots seen on shining moist surfaces. Lungs were rich in

blood and brownish red (transition between hyperaemia and pulmonary oedema) when the variations in colour were asymmetrical and uneven and the changes were distinctly localized bilaterally in the hilar regions. In the vagotomy series, brown changes bilaterally in the upper lobes and parts of the lower lobes were graded as "+"

*Frothy fluid in the air-ways* was noted by site a) the bronchi b) the trachea or c) flowing through the mouth or the orifices of the nose. As far as possible frothy fluid from pulmonary oedema was distinguished from that produced at agony or post mortem by phlegm blood, or aspirated matter. Frothy fluid has been graded in other studies (e.g. 170)

The weight of the lungs has been used by many authors in grading pulmonary changes in experimental animals (17 48 73 114 152 169 206 218 221 232 237 256)

Durlacher et al. (73) reported a lung weight of  $3.87 \pm 0.21$  and Alwall et al. (21) of  $4.0 (3.0-5.2)$  g per kg body weight in bled rabbits. Luisada (169) quoted a lung weight per kg body weight in rabbits of  $4.55 \pm 0.21$  g in normal animals and  $18.50 \pm 0.35$  g in bled animals which had received adrenaline injections. Courtice (48) found that the lung weight per kg body weight averaged about 4.90 g in normal rabbits, 5.50 g in rabbits with light pulmonary oedema, 6.30 g in those with moderate pulmonary oedema, and 8.10 g in those with severe pulmonary oedema. Stone & Loew (237) noted that in rabbits weighing 2.50 (2.00-3.00) kg and killed by a blow on the head the lung weight was  $10.58 \pm 1.41$  g. After a large dose of

adrenaline intravenously the lung-weight rose to  $21.77 \pm 7.10$  g and after phenobarbitone plus adrenaline to  $24.93 \pm 14.49$  g.

Two noteworthy arguments have been advanced against the use of the lung weight as a criterion of pulmonary oedema. Durlacher et al. (1930) found that the weight of the lungs increased after death and that with some manners of death in experimental animals it could rise twofold in 3 hours. Poolson (1934) asserted that increased lung weight could be due not only to pulmonary oedema but also to hyperaemia and pneumonia, and therefore recommended determination of the water content of the lung, since this did not increase in hyperaemia and pneumonia, whereas it did so in pulmonary oedema.

#### *Determination of the water content of the lung*

The water content of the lung has been determined by desiccation (1 170, 180 206, 212, 236, and others). Reichman (218) found that in rabbits the water content in the left lung (the right one was used for microscopical examination) in marked pulmonary oedema averaged 83.2 (80.3—89.5) % whereas in normal rabbits or those with slight pulmonary oedema the water content was 77.1 (73.8—81.3) %. Newdenhall et al. using the freeze-drying technique, noted an average water content of 79.8 % in the rabbit's lung, 83.2 % in blood, and 92.0 % in oedema fluid drying in an oven gave almost identical results. Manery et al. (1939) reported an average water content of 80.2 % in the lung and 82.5 % in the blood. Poolson (1934) found that in mice the water content of the lung averaged 77.1 % that of the blood 78.5 %, and that of oedema fluid 84.9 %. He con-

cluded that it seems evident that the water content of lungs showing a weight increase due exclusively to a increased accumulation of blood does not differ essentially from that of normal lungs. On the other hand, a weight increase caused by oedema fluid will raise considerably the water content of the lungs. He considered that the water content would hence be diagnostic in distinguishing pulmonary oedema from hyperaemia and inflammation.

According to Poolson, freeze-drying for 48 hours maintains the lung-volume unchanged, whereas heat-drying of organic matter especially that containing blood, causes loss of volume and formation of tough membranes that prevent complete drying.

The method has been criticized by several authors. Wood & Moe (1942) considered that the water content could not be regarded as a reliable criterion of oedema since the protein concentration in the oedema fluid varies markedly and since the results differ according to the length of drying and the temperature. Vlascher et al. (246) did not consider the method reliable as the blood-cell/plasma ratio was increased in the blood present in the lung. Reichman (218) on the other hand, found a close correlation between water content and the amount of pulmonary oedema observed by gross examination.

The following technique was used in the present study. The left lung was placed in an aluminium-foil beaker was cut into small pieces and desiccated under infrared light (2Kw X system Gronert infr red lamp). The heat radiation from the lamp could be regulated by means of a slide resistor. The desiccation was continued until two weighings at 3-minute intervals gave the same weight. Lung tissue cut in

very minute pieces dried more quickly than did less finely divided tissue, but the water content was the same in lungs from the same animal. The dry weight was calculated as a percentage of the original lung weight. The percentage water content of the lung was assumed to be 100 minus the percentage dry weight.

In 10 rabbits the heat radiation was continued for another 30 minutes. The lung pieces did not become charred and did not lose weight. In 28 rabbits both the right and the left lung were desiccated individually the lungs showed no gross changes. The average water content was 80.6 % for all the right lungs and 80.6 % for all the left lungs. Thus, the difference between the two lungs in the same animal averaged 0.5 (0—1.7) %. Desiccation of blood gave very varying water contents, as thick membranes formed on the surface and prevented the blood underneath from drying. The weight fell continuously during the desiccation process even after the blood had dried, because the membranes on the surface became charred. At desiccation of blood filled cut lungs charred membranes did not form and when the lungs had dried the weight did not fall in spite of continued radiation.

In choosing the method for drying of the lung (lung tissue) to constant weight the alternatives were the technically simpler heat lamp method or freeze-drying (freezing at  $-20^{\circ}\text{C}$ , drying in vacuo at  $-1^{\circ}\text{C}$  for 24 hours, and thereafter drying in vacuo at room temperature). The first mentioned method has been reported to be less

effective (211). The experiments described below were carried out with lungs from 6 normal rabbits which had been killed by bloodletting and 5 normal rabbits killed by intravenous injection of 5 ml of Narkotal solution. In the first series the lungs contained little blood and in the second series much blood.

The left lung from each animal was cut out into small pieces which were dried means of a heat lamp as described in the foregoing and the right lung was dried whole by the freeze-drying method, suspended from a thin thread, a small piece of a bronchus being kept for attachment of the thread. In the bled animals the water content of the lungs, determined by the said methods, were 80.3 (79.7—81.1) % and 70.0 (77.8—80.7) % respectively, the corresponding figures for the animals killed by injection of Narkotal were 81.2 (81.0—81.6) % and 79.0 (77.1—80.0) %.

The practical conclusions from these experiments are that highly blood filled lungs can be adequately dried by the heat lamp technique, and that the values for the water content of the lung obtained by this method are as reliable as those obtained by the freeze-drying method. Therefore, the technically simpler procedure (the heat lamp technique) was used in my experiments. It should be noted that the values were slightly higher with the heat lamp method than with the freeze-drying method, the difference is slight however and seems to be of no practical importance as far as the experimental results and the conclusions are concerned.

*Microscopical studies* of fixed sectioned and stained material have proved disappointing in experimental acute massive oedema

There is sometimes no stainable material in the alveoli. This result is not surprising because it is known that in early acute lung oedema of several types the oedema fluid may be nearly protein-free, as was shown by Koeing (1949) and others. The only early evidence of such lung oedema that is visible microscopically is interstitial-wall thickening because protein-free fluid in alveoli is undetectable in conventional histological preparations (Vlascher et al. 1946). Reichsman (1946) found that in lung oedema after bilateral vagotomy in mice the microscopical picture usually confirmed the gross examination with the exception that small and sometimes even moderate amounts of lung oedema were difficult to demonstrate under the microscope. Duracher et al. showed that in rabbits which had been dead for 3 hours or more, alveolar oedema developed after death.

The lungs of 320 rabbits were studied microscopically. The slides were examined by Ass. Prof. N. O. Berg at the Institute of Pathology. The following typical features were noted:

Normal picture (Figs. 12 and 13)

Bleeding (Figs. 14 and 15) Focal haemorrhages were a characteristic finding in many series. The haemorrhages varied in number and size and involved from a few to several hundred alveoli.

Hyperaemia (Fig. 16) was relatively rare in the bled animals, whereas all stages, from insignificant to massive hyperaemia in all the capillaries, were seen in the animals that died spontaneously.

Interstitial oedema (Fig. 17) occur

red in the form of distension of the periarterial tissue in the lung.

Bronchiolitis (Figs. 18 and 19) with more or less marked destruction of the epithelium in the bronchioles but not in the larger bronchi, was seen in the 'narkotical' series.

Purulent bronchitis and stagnation of secretion were common findings in some series and were probably due to aspiration.

Atelectasis was one of the most common changes. In many cases the changes involved only a small part of the lung.

Pneumonia was noted mainly in vagotomized animals. A slight increase of leucocytes in the alveolar walls without any other signs of pneumonia was demonstrated in the adrenaline series.

Thickening of the alveolar septa of varying degree was seen in animals with hyperaemia, pulmonary oedema, or decreased content of air in the alveoli but this finding was usually not recorded in this investigation, since the thickness of the septa often varied in the same animal.

Alveolar oedema (Figs. 20, 21, 22, 23, 24 and 25) was seen as precipitated albumin in the alveoli, in typical cases containing a few red blood-cells and alveolar phagocytes. The degree of alveolar oedema was coded as +, ++ and +++.

The lung-volume was determined in some 30 rabbits by immersion of the right lung in a beak filled with water. The overflow which discharged through a spout, was collected and weighed. The trachea and the hilum were tied so as to prevent

very minute pieces dried more quickly than did less finely divided tissue, but the water content was the same in lungs from the same animal. The dry weight was calculated as a percentage of the original lung weight. The percentage water content of the lung was assumed to be 100 minus the percentage dry weight.

In 10 rabbits the heat radiation was continued for another 30 minutes. The lung pieces did not become charred and did not lose weight. In 28 rabbits both the right and the left lung were desiccated individually the lungs showed no gross changes. The average water content was 80.0 % for all the right lungs and 80.0 % for all the left lungs. Thus, the difference between the two lungs in the same animal averaged 0.5 (0—1.7) %. Desiccation of blood gave very varying water contents as thick membranes formed on the surface and prevented the blood underneath from drying. The weight fell continuously during the desiccation process even after the blood had dried, because the membranes on the surface became charred. At desiccation of blood filled cut lungs charred membranes did not form and when the lungs had dried the weight did not fall in spite of continued radiation.

In choosing the method for drying of the lung (lung tissue) to constant weight the alternatives were the technically simpler heat lamp method or freeze-drying (freezing at  $-20^{\circ}\text{C}$ , drying in vacuo at  $-1^{\circ}\text{C}$  for 24 hours, and thereafter drying in vacuo at room temperature). The first mentioned method has been reported to be less

effective (211). The experiments described below were carried out with lungs from 5 normal rabbits which had been killed by bloodletting and 5 normal rabbits killed by intravenous injection of 5 ml of Narkotal solution. In the first series the lungs contained little blood and in the second series much blood.

The left lung from each animal was cut out into small pieces which were dried means of a heat lamp as described in the foregoing and the right lung was dried whole by the freeze-drying method, suspended from a thin thread, a small piece of a bronchus being kept for attachment of the thread. In the bled animals the water content of the lungs, determined by the said methods, were 80.3 (79.7—81.1) % and 79.0 (77.8—80.7) % respectively, the corresponding figures for the animals killed by injection of Narkotal were 81.2 (81.0—81.6) % and 79.0 (77.1—80.0) %.

The practical conclusions from these experiments are that highly blood filled lungs can be adequately dried by the heat lamp technique, and that the values for the water content of the lung obtained by this method are as reliable as those obtained by the freeze-drying method. Therefore, the technically simpler procedure (the heat lamp technique) was used in my experiments. It should be noted that the values were slightly higher with the heat lamp method than with the freeze drying method the difference is slight. However and seems to be of no practical importance as far as the experimental results and the conclusions are concerned.

## Control material

The control material consisted of 50 normal rabbits and 78 rabbits whose ureters were tied and which were killed by bloodletting 0.1 and 1, 2, 3 or 4 days, respectively after the operation. The lungs were also studied in rabbits which after ligation of the ureters were killed by other methods or allowed to die spontaneously in their uraemia. The ligation of the ureters was made bilaterally above the pubes at the bladder under anaesthesia with Xylocalin®.

*Examination of the living animals*

**Appearance and behaviour** In most cases no symptoms were noted on the first few days after ureteral ligation. Thereafter the animals became increasingly drowsy and rarely took food or drink. In those with severe uraemia trembling, twitchings, and unsteady gait were common features. Diarrhoea occurred in many cases. Marked muscle weakness intercurrent (the last few hours before death the animals lay down on their sides and did not move much. Many of them died unnoticed).

The animals lost weight, on an average about 2% when the bladder was emptied during the ureteral ligation,

and thereafter about 1% per day. A few animals gained weight on the first days after their ureters had been tied, whereas in others diarrhoea led to a weight loss of 4% or more per day. The average weight loss in uraemic rabbits is probably explained by inappetence (food and water), reduction of the gastrointestinal contents, tissue breakdown, and loss of fluid through diarrhoea.

**Laboratory data** In 37 rabbits non-protein nitrogen (NPN) in the blood (determined by Rappaport's method) rose after the ureteral ligation by about 70 (40—120) mg per 100 ml per day from about 45 (25—68) mg per 100 ml before the operation (Table 1) in the rest of the rabbits, NPN was determined only at the bloodletting. The highest recorded value in all the series was 426 mg per 100 ml in a rabbit which was killed by bloodletting after 4 days. In most cases of spontaneous death in uraemia the NPN level was about 300 mg per 100 ml. Serum potassium (determined by flame photometry) increased gradually to high values (Table 1) after 4 days of anuria; the mean value was 10.0 mEq per litre.

Respiratory rate was checked for 4



air from leaving the lung during dissection. Before the measuring procedure, the lung root was cut off close to the thread, a pair of heavy forceps was attached to the thread and the forceps and the lung were placed in the beaker.

The *diagnosis of pulmonary oedema* was considered established in the present study if at least three of the following examinations were positive: Chest X-ray, gross examination, estimation of frothy fluid in the air ways, measure-

ments of lung weight, determination of the water content in the lungs, and microscopical studies. In the vagotomy series, the three criteria of pulmonary oedema should include microscopical alveolar oedema or a water content above the upper limit of normal range. The extent to which the individual criteria vary under experimental conditions (see Chapters 6—9) will be discussed in Chapter 10.

## Control material

The control material consisted of 50 normal rabbits and 78 rabbits whose ureters were tied and which were killed by bloodletting 0.1 and 1.2, 3, or 4 days, respectively after the operation. The lungs were also studied in rabbits which after ligation of the ureters were killed by other methods or allowed to die spontaneously in their uraemia. The ligation of the ureters was made bilaterally above the pelves at the bladder under anaesthesia with Xylorcin®.

*Examination of the living animals*

**Appearance and behaviour** In most cases no symptoms were noted on the first few days after ureteral ligation. Thereafter the animals became increasingly drowsy and rarely took food or drink. In those with severe uraemia trembling, twitchings, and unsteady gait were common features. Diarrhoea occurred in many cases. Marked muscle weakness intercurrent the last few hours before death the animals lay down on their sides and did not move much. Many of them died unnoticeably.

The animals lost weight on an average about 2% when the bladder was emptied during the ureteral ligation

and thereafter about 1% per day. A few animals gained weight on the first days after their ureters had been tied, whereas in others diarrhoea led to a weight loss of 4% or more per day. The average weight loss in uraemic rabbits is probably explained by inappetence (food and water), reduction of the gastrointestinal contents, tissue breakdown, and loss of fluid through diarrhoea.

**Laboratory data** In 37 rabbits non-protein nitrogen (N.P.N.) in the blood (determined by Rappaport's method) rose after the ureteral ligation by about 70 (40—120) mg per 100 ml per day from about 45 (28—68) mg per 100 ml before the operation (Table 1) in the rest of the rabbits, N.P.N. was determined only at the bloodletting. The highest recorded value in all the series was 426 mg per 100 ml in a rabbit which was killed by bloodletting after 4 days. In most cases of spontaneous death in uraemia the N.P.N. level was about 300 mg per 100 ml. Serum-potassium (determined by flame photometry) increased gradually to high values (Table 1) after 4 days of anuria the mean value was 10.0 mEq per litre.

Respiratory rate was checked for 4

Table 1 *N.P.N. and serum potassium in rabbits 0—4 days after bilateral ligation of their ureters*

Anuria days	N.P.N. mg per 100 ml	Number rabbits	Serum potassium mEq/l	Number rabbits
0	45 (28—68)	37		
1	98 (60—128)	37		
2	175 (120—223)	34	5.8 (4.9—9.3)	18
3	275 (199—405)	32	8.9 (6.6—10.2)	8
4	330 (214—426)	13	10.0 (9.1—12.5)	7

days in 5 normal rabbits in connection with for instance, transportation weighing and electrocardiographic examination. The respiratory rate averaged 122 (72—220) per minute. When the animals were at rest and undisturbed the rate fell when they were agitated it rose to more than double the normal value. The highest respiratory rate was 430 per minute, measured by means of a thermistor in front of the nostrils.

Table 2 Respiratory rate was measured daily for 3 days in 51 rabbits whose ureters were tied. The average rate fell with increasing uraemia as a rule most markedly at an N.P.N. level exceeding 200 mg per 100 ml. In most cases respiration became gradually slower and was finally suspended.

Table 2 *Mean respiratory rate per minute for 51 rabbits 0—3 days after ligation of their ureters and estimated mean value for N.P.N.*

Anuria days	Respiratory rate per min. to mean and range	N.P.N. mg per 100 ml (estimated mean value)
0	163 (56—478)	45
1	127 (36—238)	101
2	99 (33—180)	171
3	79 (33—140)	275

Table 3 *Heart rate per minute and estimated mean value for N.P.N. in 51 rabbits 0—3 days after ligation of their ureters*

Anuria days	Heart rate per min. to mean and range	N.P.N. mg per 100 ml (estimated mean value)
0	218 (151—294)	45
1	224 (163—278)	101
2	219 (139—273)	171
3	230 (151—316)	75

The heart rate, measured by electrocardiography showed great variations. In 233 normal rabbits the average rate was 230 (125—368) beats per minute. In the 51 rabbits with tied ureters the rate altered little during 3 days of uraemia finally bradycardia was noted however (Table 3).

Electrocardiographic abnormalities became more frequent with increasing uraemia. Fig. 2 shows the most characteristic change. The positive T wave became increasingly flat and finally changed to negative. Broadening and deformation of the QRS complex, extrasystoles, arrhythmia, and bradycardia were noted in the final stage. Table 4 shows that after 3 days the T waves were absent in 10 % and negative in 25 %. In 15 rabbits with anuria for 3—4 days there was poor correlation

Table 4 *Occurrence of T-wave abnormalities in the electrocardiograms and estimated mean value for N.P.N. in 51 rabbits 0—3 days after ligation of their ureters*

Anuria day	Number of rabbits with T waves	positive	absent	negative	N.P.N. mg per 100 ml (estimated mean value)
0	51	0	0	0	45
1	51	0	0	0	101
2	43	4	4	4	171
3	33	5	18	10	275

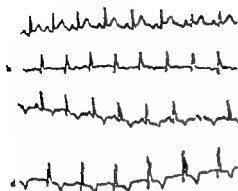


Figure 2. Electrocardiogram (lead I) in an animal with typical changes. Examination in this case carried out: a) before, b) 2 days after, c) 3 days after, d) 4 days after ligation of the ureters.

tion between the serum potassium concentration and the electrocardiographic pattern. Accordingly the electrocardiogram was normal in 2 animals with a potassium level of 10.1 mEq per litre, whereas it was greatly abnormal in another animal with a potassium concentration of 6.6 mEq per litre.

It was found that the clinical pictures, respiratory rates, and electrocardiograms of animals which had survived the first few days of anuria served as a relatively reliable basis for the estimation of remaining survival time.

Table 5. Occurrence of radiographic pulmonary changes coded as "0" and + (more severe changes did not occur) in 331 anuric rabbits 1-4 days after ligation of their ureters.

anuria day	Pulmonary changes	
	0	+
1	46	0
2	222	3
3	86	3
4	9	0

**Chest radiography** In all the rabbits of the experimental series chest X ray before ligation of the ureters showed normal lungs and normal heart size. Table 6. After ligation of the ureters slight pulmonary changes were seen within 3 days in 3 % of the animals. — Table 7. Enlarged heart shadow in most cases a slight increase, was noted in about 15 % of the severely uraemic animals.

Table 6. Occurrence of radiographically enlarged heart coded as "0" + ++ and +++ in 331 anuric rabbits 1-4 days after ligation of their ureters.

anuria day	Cardiac enlargement			
	0	+	++	+++
1	44	2	0	0
2	203	16	0	0
3	86	11	2	1
4	6	2	1	0

#### Examination immediately after death of bled animals

**Gross observation at autopsy** In all the normal rabbits the lung surfaces were smooth and pink. Among the rabbits with tied ureters no dots or spots were seen in 20 after less than 2 days of anuria. A few red dots, in rare cases small spots, were noted on the lung surfaces in 5 out of 28 rabbits (18 %) after 2 days of anuria, in 6 out of 20 rabbits (30 %) after 3 days of anuria and in 2 out of 10 (20 %) after 4 days of anuria. In severe uraemia the colour of the dots was more brown than red. In 2 severely uraemic animals the lungs were bluish pink despite the absence of a large amount of pleural effusion. In one of the latter there were

Table 1 *N.P.N. and serum potassium in rabbits 0—4 days after bilateral ligation of their ureters*

Anuria days	N.P.N. mg per 100 ml	Number of rabbits	Serum potassium mEq/l	Number of rabbits
0	45 (28—68)	37		
1	98 (65—128)	37		
2	175 (120—223)	34	5.8 (4.9—9.3)	18
3	275 (199—403)	32	8.9 (0.6—10.2)	8
4	330 (244—426)	13	10.0 (9.1—12.5)	7

days in 8 normal rabbits in connection with for instance, transportation weighing and electrocardiographic examination. The respiratory rate averaged 122 (72—220) per minute. When the animals were at rest and undisturbed, the rate fell when they were agitated it rose to more than double the normal value. The highest respiratory rate was 430 per minute, measured by means of a thermistor in front of the nostrils.

Table 2 Respiratory rate was measured daily for 3 days in 51 rabbits whose ureters were tied. The average rate fell with increasing uraemia as a rule most markedly at an NPN level exceeding 200 mg per 100 ml. In most cases respiration became gradually slower and was finally suspended.

Table 2 *Mean respiratory rate per minute for 51 rabbits 0—3 days after ligation of their ureters and estimated mean value for N.P.N.*

Anuria day	Respiratory rate per min. and range	N.P.N. mg per 100 ml (estimated mean value)
0	153 (56—428)	45
1	127 (36—238)	101
2	99 (33—180)	171
3	79 (33—140)	275

Table 3 *Heart rate per minute and estimated mean value for N.P.N. in 51 rabbits 0—3 days after ligation of their ureters*

Anuria day	Heart-rate per minute mean and range	N.P.N. mg per 100 ml (estimated mean value)
0	218 (154—204)	45
1	224 (168—278)	101
2	219 (139—273)	171
3	230 (151—310)	275

The heart rate, measured by electrocardiography showed great variations. In 233 normal rabbits the average rate was 230 (125—308) beats per minute. In the 51 rabbits with tied ureters the rate altered little during 3 days of uraemia finally bradycardia was noted, however (Table 3).

Electrocardiographic abnormalities became more frequent with increasing uraemia. Fig. 2 shows the most characteristic change. The positive T wave became increasingly flat and finally changed to negative. Broadening and deformation of the QRS complex extrasystoles arrhythmia and bradycardia were noted in the final stage. Table 4 shows that after 3 days the T waves were absent in 10 % and negative in 25 %. In 15 rabbits with anuria for 3—4 days there was poor correlation

Table 4 *Occurrence of T-wave abnormalities in the electrocardiograms and estimated mean value for N.P.N. in 51 rabbits 0—3 days after ligation of their ureters*

Anuria day	Number of rabbits with T waves	positive	absent	negative	N.P.N. mg per 100 ml (estimated mean value)
0	51	0	0	0	45
1	51	0	0	0	101
2	43	4	4	4	171
3	33	5	13	15	275

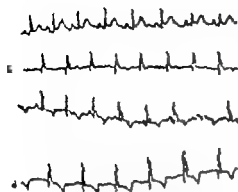


Figure 2. Electrocardiogram (lead I) in an animal with typical changes. Examination in this case carried out: a) before, b) 3 days after, c) 3 days after, d) 4 days after ligation of the ureters

tion between the serum potassium concentration and the electrocardiographic pattern. Accordingly the electrocardiogram was normal in 2 animals with a potassium level of 10.1 mEq per litre, whereas it was greatly abnormal in another animal with a potassium concentration of 6.8 mEq per litre.

It was found that the clinical picture, respiratory rates, and electrocardiograms of animals which had survived the first few days of anuria served as a relatively reliable basis for the estimation of remaining survival time.

Table 5. Occurrence of radiographic pulmonary changes coded as "0" and "+" (more severe changes did not occur) in 381 anuric rabbits 1-4 days after ligation of their ureters

Anuria days	Pulmonary changes	
	0	+
1	46	0
2	232	3
3	98	3
4	9	0

**Chest radiography** In all the rabbits of the experimental series chest X ray before ligation of the ureters showed normal lungs and normal heart size. Table 5. After ligation of the ureters slight pulmonary changes were seen within 5 days in 3 % of the animals. — Table 6. Enlarged heart shadow in most cases a slight increase, was noted in about 15 % of the severely uraemic animals.

Table 6. Occurrence of radiographically enlarged heart coded as "0" and "+" and "++" (381 anuric rabbits 1-4 days after ligation of their ureters)

Anuria days	Cardiac enlargement			
	0	+	++	+++
1	46	2	0	0
2	232	16	0	0
3	98	11	3	1
4	9	3	1	0

#### Examination immediately after death of bled animals

Gross observation at autopsy In all the normal rabbits the lung surfaces were smooth and pink. Among the rabbits with tied ureters no dots or spots were seen in 20 after less than 2 days of anuria. A few red dots, in rare cases small spots, were noted on the lung surfaces in 3 out of 28 rabbits (18 %) after 2 days of anuria, in 6 out of 20 rabbits (30 %) after 3 days of anuria, and in 3 out of 10 (20 %) after 4 days of anuria. In severe uraemia the colour of the dots was more brown than red. In 2 severely uraemic animals the lungs were bluish pink despite the absence of a large amount of pleural effusion. In one of the latter there were

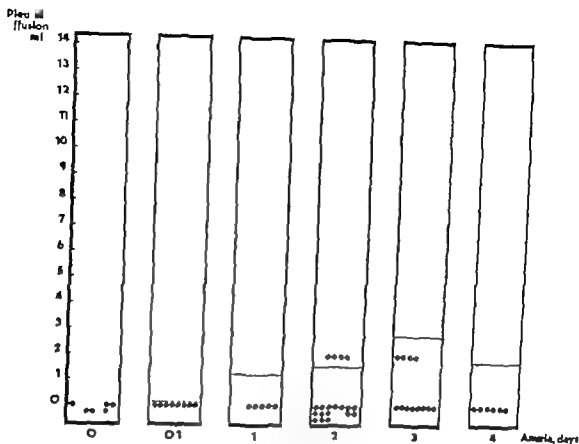


Diagram 1 Pleural effusion in normal and uraemic rabbits with anuria for 0.1 1 2, 3 and 4 days, respectively

small brown spots in the bluish red lungs.

Pleural effusion and ascites were not seen in normal rabbits, excepting 2 which had 2 ml of pleural fluid each. Most of the severely uraemic rabbits had only slight pleural effusion (Diagram 1). Among rabbits with anuria for at least 2 days pleural effusion was noted in about 45 % the amount being 3 (1—14) ml.

The amount of ascitic fluid increased markedly with increasing uraemia (Diagram 2). After 3 or 4 days of uraemia 30 rabbits had, on an average, 108 (0—355) ml of ascitic fluid. Thus, at increasing uraemia in rabbits with

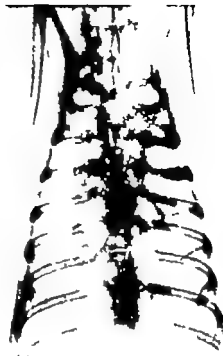
lied ureters, there was a shifting of fluid from the tissues to the abdominal cavity possibly because of the higher osmolality in the latter (the V.P.N. level in the ascitic fluid was, on an average, 10 % higher than in blood).

Pericardial effusion occurred in all the rabbits the normal ones as well. The amount was difficult to measure but in normal rabbits it could be estimated at 0.5 (0.1—1.5) ml. In severe uraemia the amount seemed to increase to about 2 ml the highest amount measured was 4 ml.

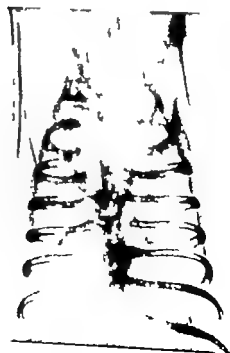
Frothy fluid in the air ways was not found, either in normal or in uraemic controls.



3



4



5

Figure 3 A normal rabbit

Figure 4 Pulmonary changes coded as +  
(light pulmonary edema)

Figure 5 Pulmonary changes coded as ++  
(moderate pulmonary edema)



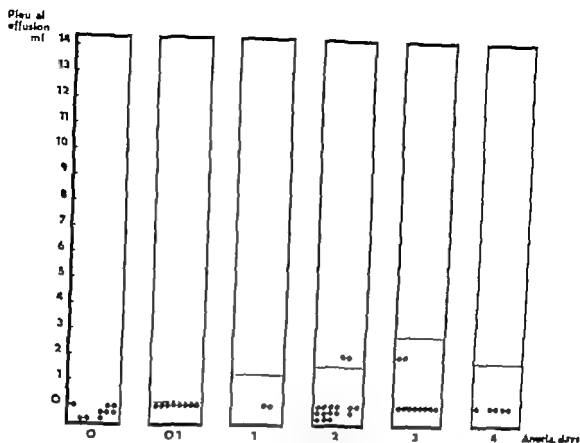


Diagram 1 Pleural effusion in normal and uraemic rabbits with anuria for 0.1 1 2, 3 and 4 days respectively

small brown spots in the bluish red lungs

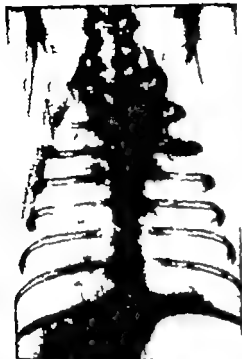
Pleural effusion and ascites were not seen in normal rabbits, excepting 2 which had 2 ml of pleural fluid each. Most of the severely uraemic rabbits had only slight pleural effusion (Diagram 1). Among rabbits with anuria for at least 2 days pleural effusion was noted in about 45 % the amount being 3 (1—14) ml.

The amount of ascitic fluid increased markedly with increasing uraemia (Diagram 2). After 3 or 4 days of uraemia 30 rabbits had, on an average 108 (2—355) ml of ascitic fluid. Thus, at increasing uraemia in rabbits with

tiled ureters, there was a shifting of fluid from the tissues to the abdominal cavity possibly because of the higher osmolality in the latter (the VPM level in the ascitic fluid was on an average, 10 % higher than in blood).

Pericardial effusion occurred in all the rabbits, the normal ones as well. The amount was difficult to measure but in normal rabbits it could be estimated at 0.5 (0.1—1.5) ml. In severe uraemia the amount seemed to increase to about 2 ml the highest amount measured was 4 ml.

Frothy fluid in the air ways was not found, either in normal or in uraemic controls.



9



10

11



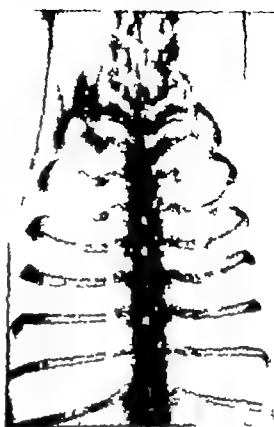
Figure 9 Atelectasis in case of profuse pleural effusion

Figure 10 Bleeding in the lung parenchyma following infarction of dextrin

Figure 11 Cardiac enlargement ("+++") without pulmonary changes



6



7



Figure 6. Pulmonary changes coded as  
+++ (severe pulmonary oedema)  
Figure 7. Pulmonary changes coded as  
+++ (severe pulmonary oedema)  
Figure 8. Pneumonia in a vagotomized rabbit.



Figure 11. Minor haemorrhage in the lungs of uraemic rabbit. Haematoxylin-eosin.  $\times 60$ .

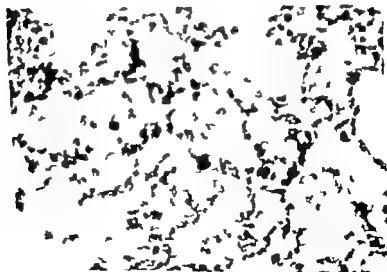


Figure 12. Profuse haemorrhages in the lungs without alveolar oedema in rabbit with severe pulmonary oedema following infusion of dextran. Haematoxylin-eosin.  $\times 400$ .

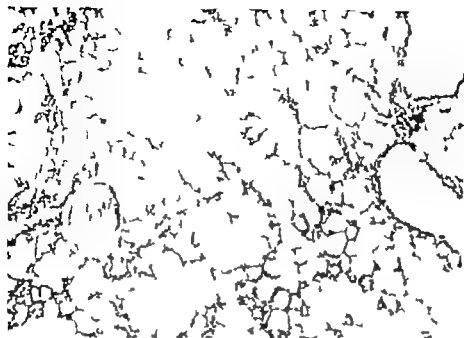


Figure 12 Normal picture of the lungs of a highly uraemic rabbit. Note the stretched alveolar wall and rounded alveoles. Haematoxylin-eosin.  $\times 63$ .



Figure 13. Normal picture of the lungs of a highly uraemic rabbit. Note the stretched alveolar wall and rounded alveoles. Haematoxylin-eosin.  $\times 100$ .

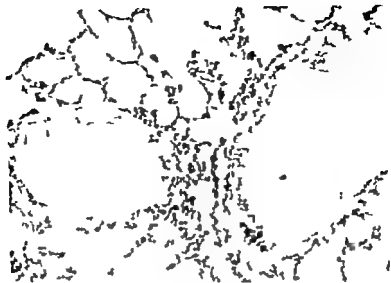


Figure 18. Bronchiolitis in uremic rabbit anaesthetized with "Narkotal" Haematoxylin-eosin  $\times 100$ .

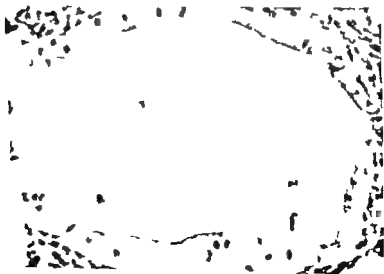


Figure 19. Bronchiolitis in uremic rabbit anaesthetized with "Narkotal" Haematoxylin-eosin.  $\times 400$ .



Figure 16 Marked hyperaemia without alveolar oedema in a rabbit with severe pulmonary oedema following injection of adrenaline. Haematoxylin-eosin.  $\times 100$



Fig. 17 Perivascular interstitial oedema in a rabbit without pulmonary oedema following injection of adrenaline. There are also minor haemorrhages. Haematoxylin-eosin.  $\times 25$

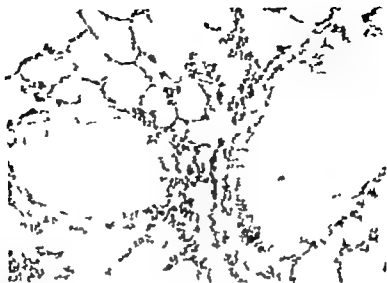


Figure 18. Bronchioles in uremic rabbit anesthetized with "Aarkotal" Haematoxylin-eosin  $\times 100$ .



Figure 19. Bronchioles in uremic rabbit anesthetized with "Aarkotal" Haematoxylin-eosin.  $\times 400$ .





Figure 20 Alveolar oedema coded as +++ in a rat bft after injection of a trenaline Hs maloxylin resin.  $\times 160$

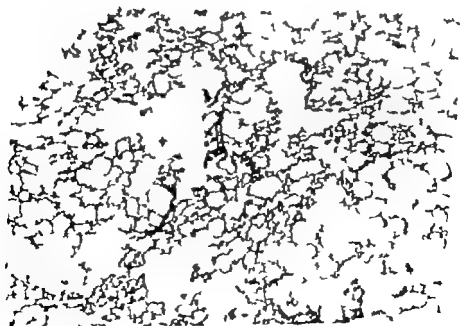


Figure 21 Alveolar oedema in rat and the rat bft coded as ++ in a g tomized rat bft Hs maloxylin resin.  $\times 160$

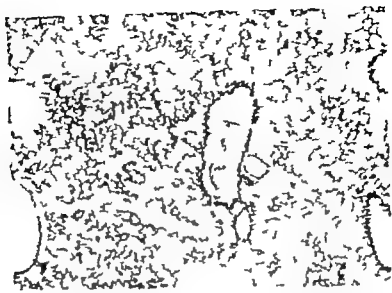


Figure 22 Focal fibrinous (irregular) pleural oedema coded as ++ in rabbit after barbitol anaesthesia. Haematoxylin-eosin  $\times 63$ .

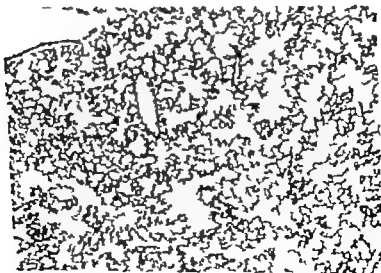


Figure 23 Generalized fibrinous (irregular) pleural oedema coded as ++ in rabbit after barbitol anaesthesia and overhydration with Ringer solution. Haematoxylin-eosin  $\times 63$ .

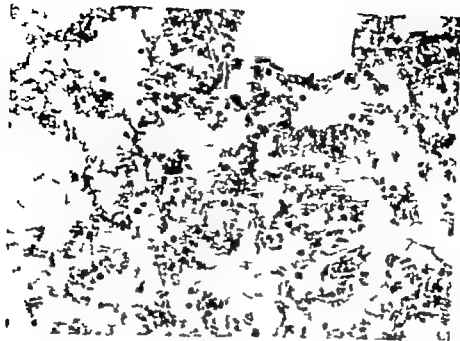


Figure 90 Adrenal cortex coded as +++ in a rabbit after injection of adrenaline. Haematoxylin-eosin.  $\times 100$

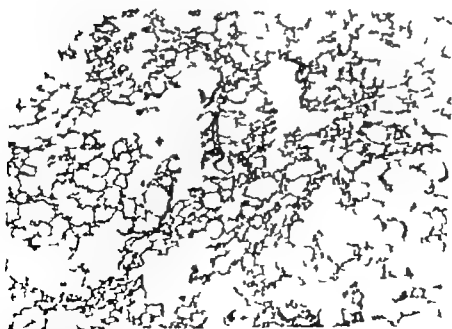


Figure 1 Adrenal cortex, hyperplasia and distinct nodules ++ in a gonotomized rabbit. Haematoxylin-eosin.  $\times 100$

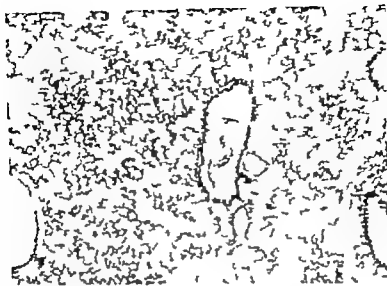


Figure 22. Focal fibrinous (thin) alveolar oedema coded as ++ in rabbit after "Xarkotal" anaesthesia. II emat xylm-cosm. X63.

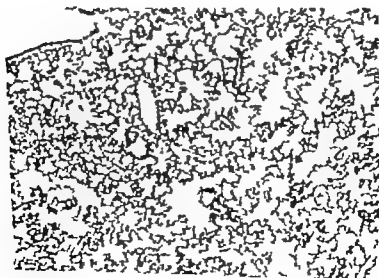


Figure 23. Generalized thin fibrinous ("stringy") alveolar oedema coded as ++ in rabbit after "Xarkotal" anaesthesia and overhydration in Ringer solution. Haematoxylin-cosm. X63.

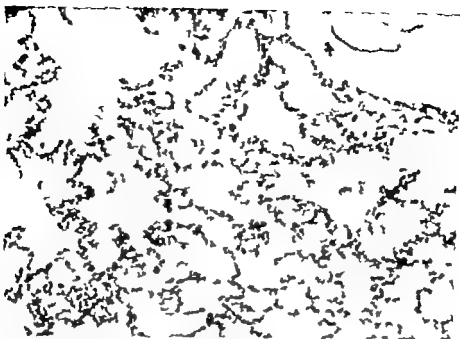


Figure 24 Generalized thin fibrinous (stringy) alveolar oedema coded as ++ in a rabbit after barbitol anaesthesia and overhydration with Ringer's solution Haematoxylin-eosin  $\times 100$

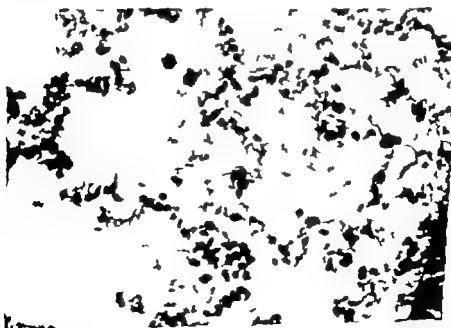


Figure 25 Alveolar oedema coded as + in a rabbit after overhydration (infusion of dextran 10%) Haematoxylin-eosin  $\times 100$

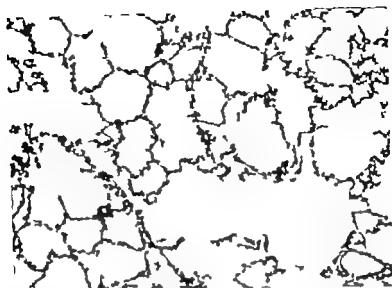


Figure 26 Insignificant microscopic pulmonary changes in an overhydrated vagotomized rabbit, in spite of four other criteria of marked pulmonary oedema. Note the plicated alveolar walls. Haematoxylin-eosin.  $\times 56$ .

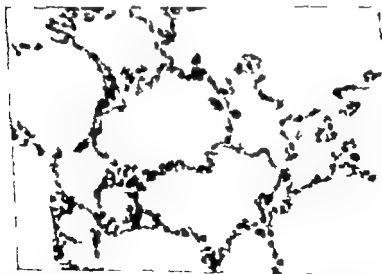


Figure 27 Insignificant microscopic pulmonary changes in an overhydrated vagotomized rabbit, in spite of four other criteria of marked pulmonary oedema. Note the plicated alveolar walls. Haematoxylin-eosin.  $\times 400$ .

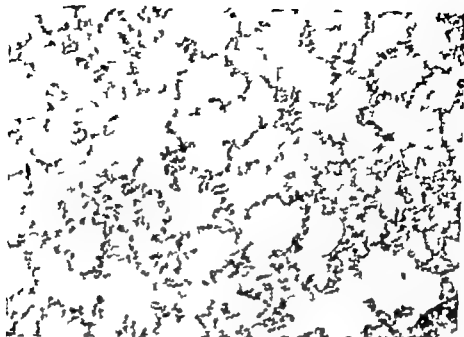


Figure 28 Postmortal pulmonary oedema. Rounded air bubbles are seen here and there in the oedema fluid. Haematoxylin-eosin  $\times 160$

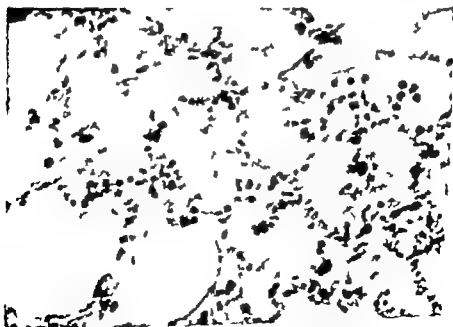


Figure 29 Postmortal pulmonary oedema. Rounded air bubbles are seen here and there in the oedema fluid. Haematoxylin-eosin.  $\times 400$ .

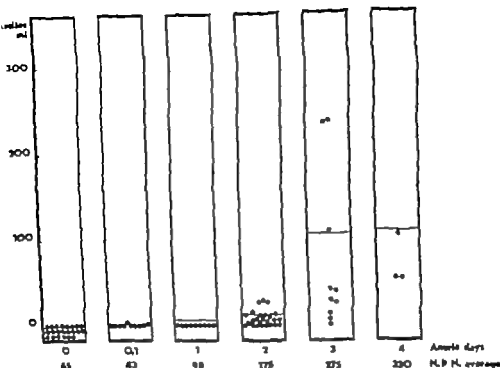


Diagram 2 Amounts of ascitic fluid in normal and uraemic rabbits with ascites for 0, 1, 2, 3, and 4 days, respectively

Lung weight (a) Relation between lung weight and body weight (Diagram 3). The average weight of the lungs of 59 bled rabbits (body weight 1.38—3.87 kg) was  $10.20 \text{ g} \pm 23.5\%$  or expressed as lung weight per kg body weight,  $3.93 \text{ g} \pm 14.7\%$ . It will be seen from the diagram that the relative value for the lung weight per kg body weight fall as the body weight increases. The animals in an experimental series should therefore have about the same weight.

Table 7 shows that in 22 bled normal rabbit weighing 2.20—2.40 kg, the lung weight was  $4.17 \pm 0.53 \text{ g per}$

kg body weight or expressed as normal value with  $\sigma / \sigma$  2.35—5.49 g per kg body weight.

(b) Relation between lung weight and degree of uraemia (Tables 7 and 8 and Diagram 4). The lung weight of animals which were bled to death at various degrees of uraemia does not differ from that in normal animals, except in a few cases. For 78 uraemic animals the mean value was  $4.25 \pm 0.45 \text{ g per kg body weight}$ . The highest value, 5.67 g per kg body weight, was recorded for a rabbit with numerous small haemorrhages in the lungs.



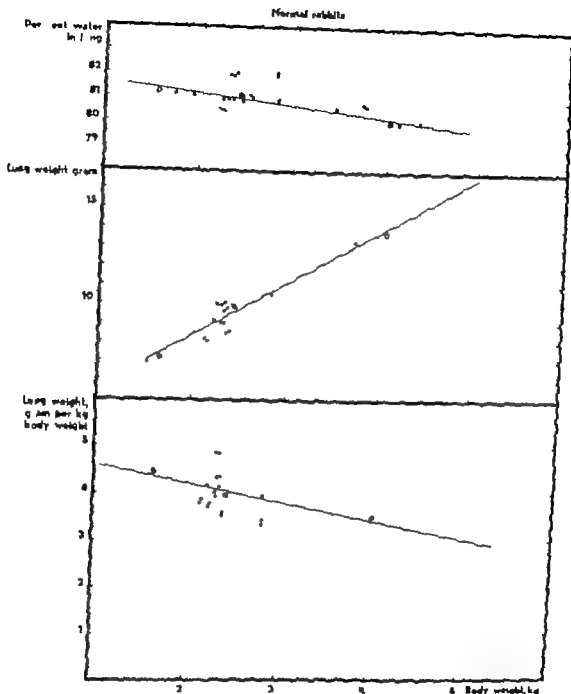


Diagram 3. Water content in the lung, lung weight, and lung weight per kg body weight in normal rabbits weighing 1.38—5.87 kg

The water content of the lung (a) lung expressed as a percentage of the Relation between the water content of lung weight varied in 59 bled normal the lung and the body weight (Diagram 3) The water content of the left rabbits (weighing 1.38—5.87 kg) between 78.4 and 82.8 % The lungs of

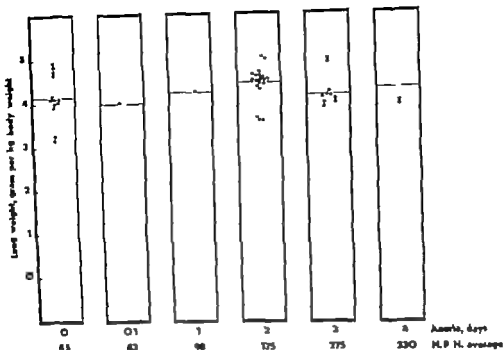


Diagram 4. Lung-weight of normal and uremic rabbits with anuria for 0.1, 1, 2, 3, and 4 days, respectively

young animals with a low body weight had a slightly higher water content than those of older animals with a higher body weight. In 22 bled normal rabbits (weighing 2.20—2.49 kg) the water content of the left lung was  $81.0 \pm 0.9\%$  or expressed as normal value

with  $2 / \sigma$ , 78.7—83.3 % (Table 7)

(b) Relationship between the water content of the lungs and the degree of uraemia (Diagram 5 and Table 7) The water content of the lungs in uraemic animals was within the normal range

Table 7 Lung-weight in g per kg body-weight water content as a percentage number / normal animals, bled uraemic non-overhydrated animals overhydrated animal and spontaneously dead non-right uraemic animals. The values are means with  $2 / \sigma$  standard deviation. The animal weight at the beginning of the experiment ranged from 2.20 to 2.49 kg

	Lung weight g per kg body-weight	Water content in the lung	Number of rabbits
Bled, normal	4.17 (2.83—5.49)	81.0 (78.7—83.3)	22
Bled, uraemic	4.25 (3.13—5.38)	80.3 (77.1—83.6)	78
Bled, uraemic, overhydrated	4.39 (3.16—5.63)	82.2 (79.2—85.2)	82
Spontaneously dead, uraemic	4.98 (3.38—6.58)	80.9 (77.1—84.7)	30

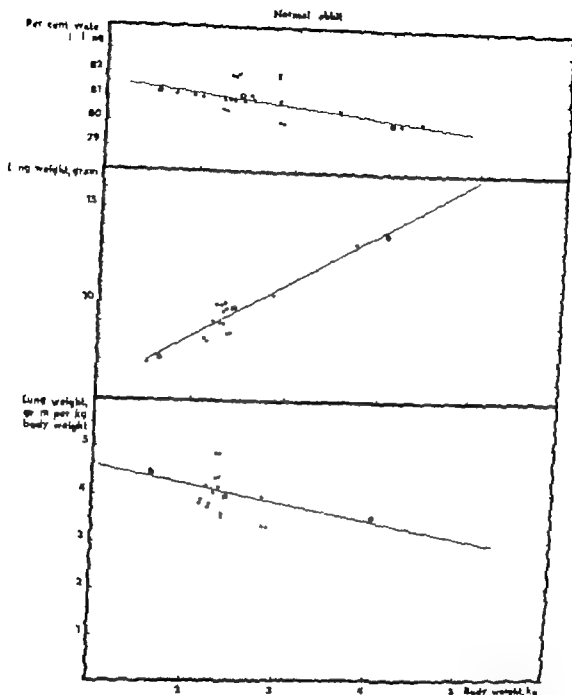


Diagram 3. Water content in the lung, lung weight, and lung weight per kg body weight in normal rabbits weighing 1.38—5.87 kg.

The water content of the lung (a) Relation between the water content of the lung and the body weight (Diagram 3) The water content of the left lung expressed as a percentage of the lung weight varied in 59 bled normal rabbits (weighing 1.38—5.87 kg) between 78.4 and 82.8 % The lungs of

Table 9 Lung-weight in g per kg body-weight, water content as a percentage the colour of the lung surfaces at gross observation post mortem, and microscopical findings in the 8 rabbits which had the highest lung-weights among 30 spontaneously dead non-rigid rabbits

Lung-weight g per kg body-weight	Water content in lung %	Colour of lung surface at gross examination	Microscopical findings	Autopsy minutes after death
8.76	82.1	Dark pink	Hyperaemia, small areas of atelectasis	207
8.65	82.2	Dark pink	Hyperaemia, small areas of atelectasis, insignificant alveolar oedema	90
8.24	84.6	Dark red	Hyperaemia, insignificant alveolar oedema	25
8.82	79.8	Purple- dark-pink	Hyperaemia, small areas of atelectasis, insignificant alveolar oedema	80
8.72	82.9	Purple pink	Hyperaemia, small areas of atelectasis	90
8.44	82.1	Dark pink	Slight hyperaemia	10

ter 3 or 4 days, the picture was normal (Figs. 12 and 13). Three of the rabbits had focal haemorrhages (Fig. 14) which at the gross examination were seen as red spots on the lung surfaces. In 1 rabbit with bluish pink lungs, atelectasis was noted. Bronchiolitis and interstitial oedema were not demonstrated.

*Examination after death of animals dying spontaneously without rigor mortis*

39 rabbit died spontaneously in their uremia 81 (45—147) hours after ureteral ligation, at a mean  $\text{NPN}$  level of 533 (205—444) mg per 100 ml, and were examined while their bodies were still warm and soft. a) 12 rabbits were examined within 10 minutes of death they had pink lung surfaces

without pulmonary changes, b) 16 rabbits were examined within 10—80 minutes, and c) 11 within 90 minutes of death.

**Gross examination** The colour of the lung surfaces varied between pale pink, bluish-purple pink, pale red dark red, or intermediate shades. It will be seen from Table 9 that only a few animals had dark red heavy lungs. Red or brown dots were present as frequently as in the bled animals. Spotted or mottled surfaces were not seen.

**Frothy fluid in the air ways** was not demonstrated in any animal.

**Lung weight** (a) In 12 animals examined within 10 minutes of death the mean lung weight was 4.57 (3.95—5.44) g per kg body weight. In 16 examined within 10—80 minutes of

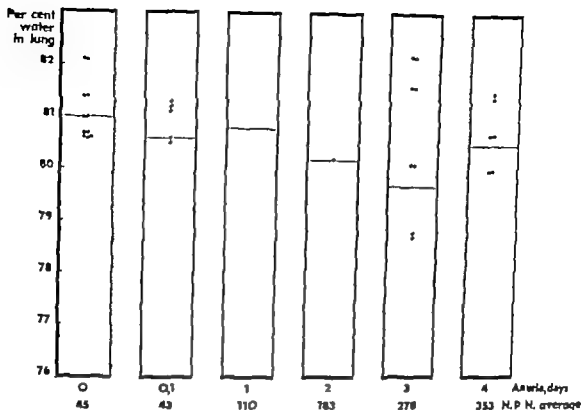


Diagram 5 Water content in the lungs of normal and uremic rabbits with anuria for 0.1, 1, 2, 3 and 4 days, respectively

Table 8 Lung-weight in g and g per kg body weight in bled normal animals and animals which had been anuric for 0.1—4 days before bloodletting. The animals' weights at the beginning of the experiment ranged from 2.20 to 2.49 kg

	Number of rabbits	Anuria, days	Lung-weight g mean	Lung weight g per kg body weight
Normal	22	0	9.70	4.17
Uraemic	10	0.1	9.23	4.06
	10	1	9.80	4.36
	28	2	10.40	4.02
	20	3	9.47	4.27
	10	4	9.79	4.58
Mean	8		9.81	4.42

in 86 % and below the lower limit for normal in 15 %. The mean water content was  $80.3 \pm 1.3$  % that is, slightly lower in the uraemic animals than in normals. The difference is significant. Rabbits with anuria for 4 days had a normal water content in their lungs, which suggests that uraemia in itself does not lead to a low content of water in the lungs but that this is due to other factors. True fluid depletion can be caused by, for instance diarrhoea and relative depletion by collection of large amounts of ascitic fluid.

Microscopical examination of the lung tissue. In 12 out of 15 rabbits which were killed by bloodletting at

death, the mean lung weight was  $7.71 \pm 2.80$  g per kg body weight. In 30 bled rabbits with anuria for at least 3 days, the mean lung weight measured immediately after death was  $4.19 \pm 0.4$  g per kg body weight, and in 39 rabbits which died spontaneously in uraemia and were examined within 1 / hours of death, it was  $4.98 \pm 0.64$  g per kg body weight. The difference between the first and the last two series is significant. Hence large amounts of fluid (blood or serum) must be transported to the lungs during the first 24 hours after death. These findings thus agree with those obtained by Durlacher et al. (73) under different experimental conditions.

The water content in the lungs. In 21 rabbits which died spontaneously in uraemia and were examined within 24 hours of death, the mean content of water in the lungs was  $82.6 \pm 2.5\%$ . For comparison may be used 39 rabbits (see section 2 above) which died spontaneously in uraemia and were examined within 1 / hours of death, with a mean of  $80.6 \pm 1.3\%$  and 30 bled severely uraemic rabbits with anuria for at least 3 days in which the mean value was  $81.2 \pm 1.2\%$ . The difference is significant. The increase of the water content in the lungs after death, which in comparison with the increase of the lung weight is relatively moderate, and the protein-rich alveolar oedema (see below) suggest that after death blood-cells and proteins are transported to the lungs, whereas the water conveyed to the lungs is less than in pulmonary oedema *in vivo*.

**Micronscopical examination.** In 13

out of 14 rabbits which died spontaneously and were examined within 24 hours of death, profuse to moderate alveolar oedema was present even in those whose lungs weighed only 6—7 g per kg body weight. The alveoli contained large amounts of precipitated protein. Hyperaemia in the alveolar walls was typical, whilst red blood cells and alveolar phagocytes were rarely seen in the alveoli.

The reason why postmortal pulmonary oedema in these experiments occurred to such varying extent is not clear. The position of the body is an important factor in that the lowest parts of the lungs were always most oedematous. Overhydrated rabbits which were examined a long time after death had high lung weights. Other factors, for instance the rate at which the blood coagulated after death, may possibly have played a part. In this study only the significance of uraemia has been considered. Durlacher's et al. experiments have shown that the manner of death is a factor of importance.

The following experiments were made in order to throw further light on the problem.

10 normal and 8 severely uraemic rabbits were killed by intravenous injection of 10 ml of air X-ray (6 rabbits) showed post mortally no abnormalities in the lungs but cardiac enlargement. Autopsy was performed on 11 of the 18 animals immediately after death. None had frothy fluid in the airways. In 2 normal animals there was gross evidence of marked hyperaemia. The water content of the lungs was  $81.4$  ( $80.3$ — $82.5$ )%. The lung weight was of the normal rabbits  $5.25$  ( $4.75$ — $5.00$ ) and

death it was  $5.15$  ( $4.01-6.76$ ) g and in 11 examined within 90 minutes of death  $5.19$  ( $4.12-6.55$ ) g per kg body weight. Table 9 shows data for the animals with the highest lung weights. Table 7. In all the 39 rabbits the mean lung weight was  $4.98 \pm 0.64$  g per kg body weight or expressed with  $2\frac{1}{2}$  sigma  $3.38 \pm 6.58$  g per kg body weight. These figures have been chosen as "normal values" for spontaneously dead rabbits in these studies.

(b) The water content of the lungs. In the 12 rabbits in which the measurements could be started within 10 minutes of death the mean water content of the lungs was  $81.0$  ( $80.3-82.7$ ) %. In rabbits examined within 11-80 minutes of death the value was  $80.7$  ( $78.2-84.6$ ) % and in 11 rabbits examined within 90 minutes of death it was  $81.0$  ( $79.0-83.2$ ) %. In 39 rabbits which died spontaneously in their uraemia (Table 7) the mean water content of the lungs was  $80.9 \pm 1.5$  % or expressed with  $2\frac{1}{2}$  sigma,  $77.1-84.7$  %.

*Microscopical examination.* In some animals small areas of atelectasis were seen, but in most cases the findings did not differ from those in the bled animals. There were some exceptions, namely rabbits with a high lung weight (Table 9) which exhibited hyperaemia and, in all cases, oedema in a few alveoli as well.

#### *Examination 24 hours after death of animals with rigor mortis*

21 rabbits which died spontaneously in uraemia, 16 normal rabbits, and 15 rabbits with severe uraemia which were

killed by intravenous injection of 10 ml of air were examined after death. At observation of the first mentioned group rigor mortis was found to begin in the neck about  $1\frac{1}{2}$  hours after death.

*Gross observation.* The lungs of the rabbits were either bright red and light, or heavy and dark red to bluish purple with diverse mottled markings. In those of the latter appearance the picture resembled that seen after death in rabbits with pulmonary oedema. The lung markings were distinguishable from pulmonary oedema in that they were more symmetrical and extended peripherally and into all the lobes, but the lung surfaces were in these cases so markedly changed that it was impossible to diagnose any slight or moderately severe changes that could have been present in vivo. Thus, in rabbits which had been dead for 24 hours, pulmonary oedema could not be diagnosed but in many cases its presence could be excluded.

*Frothy fluid in the air ways.* In 4 out of 21 rabbits (19 %) which had died spontaneously in their uraemia and in 6 out of 31 (19 %) which had been killed by intravenous injection of air frothy fluid was demonstrated in the air ways. On the other hand, in 29 animals which had died in uraemia and 20 which had been killed by injection of air no frothy fluid was found at examination while their bodies were still warm and soft. This would suggest that frothy fluid in the air ways can form post mortem.

*Lung weight.* In 21 rabbits which died spontaneously in their uraemia and were examined on the day after

## Symptoms and pulmonary oedema at overhydration

The experiments comprise the following series. I. Increase of the blood volume: 1) blood transfusions, 2) infusion of dextran solution. II. Increase of the extracellular fluid: 1) infusion of saline, 2) infusion of mannitol solution. III. Increase of all the fluid spaces: 1) infusion of glucose solution, 2) infusion of urea solution.

*Increase of blood volume**Blood transfusion*

The first known blood transfusion was given to Pope Innocentius VIII in 1492. The three donors died and the Pope became severely ill. Lower (1885) performed the first successful transfusion in animals. It was only after Landsteiner's discovery of the blood-groups in 1901 that blood transfusion was widely used therapeutically.

Pulmonary oedema after blood transfusion in man has been described by several authors (57, 58, 67, 103, 122, 141, 191, 221), notably in uncompensated heart failure, bronchopneumitis, chronic anaemia, massive haemorrhage, and severe infection.

Shaper, Shafer & Wallace (1942) transfused into normal subjects up to 5000 ml of blood, serum, or isotonic saline for about ½ hour. The mean pressure rose to a most 11 cm H<sub>2</sub>O but fell rapidly except after transfusion of blood. Chest X

ray showed distinct lung densities at the hill and slight cardiac enlargement. Vital capacity decreased. Electrocardiography showed abnormalities indicating slight right heart stress.

Müller (1874) gave infusions of blood to dogs intravenously up to 17% of the body-weight for 1–3 hours. No pulmonary oedema was noted, but hyperaemia, numerous small haemorrhages, and exudate in the pleural cavities and the abdominal cavity occurred. In experiments with blood transfusions in dogs carried out by Cohnheim & Liebhaf (1877) pulmonary oedema failed to appear in most cases, but numerous punctiform haemorrhages in the lungs arose. Krumpholtz & Chazotte (1922) increased the total red-cell volume to three times the normal by daily blood transfusions (the plasma volume, on the other hand, decreased). Pulmonary oedema did not develop, not even in a dog which lived for 40 days with twice the normal blood volume. Gibson & Gibson (1942) reduced the lung tissue in cats by lobectomy; these animals developed pulmonary oedema after one transfusion of a blood volume that was well tolerated by normal animals. In the presence of pulmonary oedema the pressure in the pulmonary artery was high but that in the left atrium normal. Loebson & Karnoff (1946) administered large amounts of isotonic saline solution, Tyrode solution, borin albumin-salt solution, and blood separately in the carotid arteries. The total amount of injected fluid was 1–3 times the animal's estimated blood volume. The infusion was performed within half



for the uraemic ones 4.15 (3.66—5.47) g per kg body weight.

16 normal rabbits and 16 rabbits with severe uraemia were killed by injection of 10 ml of air intravenously and examined within 24 hours of death. In the normal rabbits the mean lung weight was  $6.29 \pm 1.06$  g per kg body weight and in the uraemic rabbits it was  $7.00 \pm 1.51$  g per kg body weight. The difference is not significant. The mean water content of the lungs was for the normal animals  $81.8 \pm 1.4$  % and for the uraemic animals  $83.7 \pm 1.9$  %. The difference is not significant.

Examination by this technique did not show that the tendency to pulmonary oedema after death was significantly higher in uraemic than in non uraemic rabbits.

#### *Uraemia and pulmonary oedema*

In rabbits that died in uraemia, pulmonary oedema was not present at autopsy immediately after death. The tendency to pulmonary oedema appearing after death was not significantly higher in uraemic than in non uraemic rabbits.

*Survey* | See page 145

## Symptoms and pulmonary oedema at overhydration

The experiments comprise the following series I. Increase of the blood volume 1) blood transfusions, 2) infusion of dextran solution II. Increase of the extracellular fluid 1) Infusion of saline 2) Infusion of mannitol solution III. Increase of all the fluid spaces 1) infusion of glucose solution, 2) infusion of urea solution.

*Increase of blood volume***Blood transfusion**

The first known blood transfusion was given to Pope Innocentius VIII in 1492. The third day he died and the Pope became severely ill. Lower (1868) performed the first successful transfusion in animals. It was only after Landsteiner's discovery of the blood-groups in 1901 that blood transfusion was widely used therapeutically.

Pulmonary oedema after blood transfusion in man has been described by several authors (57 58, 67 103 132, 141 191 228); notable in uncompensated heart failure, bronchopneumonia, chronic anaemia, nervous haemorrhage and severe liver damage.

Sharpey Schafer & Wallace (1912) infused into normal subject up to 2000 ml of blood, serum, or isotonic saline for about ½ hour. The venous pressure rose to, at most, 11 cm H<sub>2</sub>O but fell rapidly except after transfusion of blood. Chest X

ray showed distinct lung densities at the hilum and slight cardiac enlargement. Vital capacity decreased. Electrocardiography showed abnormalities indicating slight right heart stress.

Müller (1874) gave infusions of blood to dogs intravenously up to 17 % of the body weight for 1–2 hours. No pulmonary oedema was noted, but hyperaemia, numerous small haemorrhages, and exudate in the pleural cavities and the abdominal cavity occurred. In experiments with blood transfusions in dogs carried out by Cohnheim & Liehtheim (1877) pulmonary oedema failed to appear in most cases, but numerous punctiform haemorrhages in the lungs arose. Krumpholtz & Chassin (1922) increased the total red-cell volume (three times the normal by daily blood transfusions the plasma volume on the other hand decreased pulmonary oedema did not develop, not even in a dog which lived for 40 days with twice the normal blood volume. Gibbon & Gibbon (1942) reduced the lung tissue in cats by lobectomy these animals developed pulmonary oedema after one transfusion of a blood volume that was well tolerated by normal animals. In the presence of pulmonary oedema the pressure in the pulmonary artery was high but that in the left trunk normal. Lebeda & Sarnoff (1946) administered large amounts of isotonic saline solution, Tyrode solution, bovine albumin-salt solution, and blood separately in the carotid arteries. The total amount of injected fluid was 1–3 times the animal's estimated blood volume. The infusion was performed within half

an hour. The incidence of pulmonary oedema was highest after infusion of blood, lower after albumin salt solution, and lowest after saline solution. Huckabee et al. (1950) induced hypervolaemia in 10 dogs by intravenous infusion of blood or albumin plus fluid to decompensation. In some cases more than 20 % of the body weight was given for  $\frac{1}{2}$  to 2 hours. Pulmonary oedema was produced more readily by quick than by slow infusion more readily with 10 % than with 5 % albumin solution, and the incidence was lowest after infusion of whole blood. Young et al. (1955) gave dogs blood transfusions to almost double the original blood volume at a rate of 1 ml per minute and kg pulmonary oedema did not develop; the pressure in the right atrium did not rise.

Aikawa (1950) found that the blood volume in the rabbit was 70 % of the body weight. Jung (1958) quotes a value of 5 % and Drenckhahn (1958) 7.7 %.

### *Own investigations*

In 21 uraemic rabbits, blood in amounts equalling 12 % of the animals' body weights was transfused during 1  $\frac{1}{2}$  hours. In 10 of the rabbits the ureters were tied 48 hours before the transfusion and in 11 the operation was made immediately before the transfusion. The animals were killed by bloodletting about 4  $\frac{1}{2}$  hours after the completion of the transfusion.

The transfused volume and the time of bloodletting were the same as in the experiments with dextran described under heading 2.

Blood for transfusion was obtained by cardiac puncture from 5 donors. The blood was mixed and 10 mg of heparin

Performed at the Blood Bank of the University Hospital, Lund, Sweden. (Head Bengt Löw)

were added. Blood samples for cross tests of the donors' blood-corpuscles and the recipients' serum were drawn from the ears (donors) and the heart (recipients). Cross matching at 37 °C was done at least 1 week, on an average 15 days, before the transfusion. Incompatibility was found in 7 % of the tests.

### *Symptoms in vivo*

**Appearance and behaviour.** In most cases exophthalmos developed after the transfusion, but no or only slight dyspnoea was noted. The animals were slow in reacting, moved very little, and trembled slightly, but their muscular strength was good. The degree of uraemia seemed to have little influence on the symptoms. Convulsions and myasthenia were not noted.

The respiratory rate fell during the transfusion from 170 (88—200) to 110 (80—208) per minute. Immediately before the bloodletting it was 125 (72—180) per minute. The more highly uraemic animals had a lower respiratory rate.

The heart rate was 239 (170—308) and 230 (91—527) beats per minute before and after the transfusion, respectively. Immediately before the bloodletting the rate was 233 (120—316) beats per minute. The difference was small between slightly and moderately uraemic animals. The highest value (522) was recorded for a rabbit with ventricular fibrillation.

Electrocardiographic abnormalities occurred in 7 out of 11 slightly uraemic animals and persisted or were accentuated in 6 out of 10 moderately uraemic animals during the transfusion.

alone. At the completion of transfusion 8 out of 21 rabbits had normal electrocardiograms, 4 had flat T waves, 3 had negative T waves, and 6 had severe disturbances, such as arrhythmia, deformation of the QRS-complex, ventricular fibrillation, etc. At the bloodletting  $4\frac{1}{2}$  hours later the electrocardiograms were in 9 cases as before, the changes had deteriorated in 5, and improved in 7. The 3 rabbits with evidence of pulmonary oedema had after the transfusion a normal electrocardiogram, flat T waves, and ventricular fibrillation, respectively. In the last mentioned case improvement was noted the other two deteriorated up to the bloodletting and thereafter the T waves were flat and negative, respectively.

Marked pulmonary changes appeared in the radiogram (3 of 21 films were spoiled) after the transfusion in 1 rabbit slight changes were seen in 6, whereas the picture was still normal in 11. Immediately before the bloodletting (Table 10) 2 out of 21 rabbits had marked radiographic changes (both had at autopsy heavy pleural haemorrhage 75 ml and 91 ml, respectively) and 4 had slight changes. At autopsy after bloodletting pulmonary oedema evidenced by three criteria was noted in 1 of the last named 4 animals, whilst no pulmonary changes were found in the other 3. Severe hyperaemia could possibly have produced some of the above-mentioned radiographic pulmonary changes.

The radiographic heart size was before the transfusion slightly increased in 1 case. After the transfusion it was

normal in 7, slightly increased in 8, moderately increased in 2, and non-assessable in 4 cases. Immediately before the bloodletting (Table 10) the heart was of normal size in 5, slightly enlarged in 8, moderately enlarged in 5 and non-assessable in 3 rabbits. In no animal did the heart size decrease during the period after the transfusion. No difference could be established between slightly and moderately uraemic rabbits.

#### *Postmortem findings*

On cutting the carotid artery at the bloodletting, about twice as much blood as the usual amount, 84 (30—110) ml, spurted from the artery under high pressure the average being 127 (80—180) ml. In 2 rabbits large amounts of bloody fluid were found in the pleural cavities. Eight rabbits had highly red-coloured and 4 lightly red-coloured ascitic fluid. At autopsy minor subcutaneous haemorrhages were seen, but there was no melæna. On the lung surfaces were seen in 4 cases a few and in 3 cases numerous red dots, in 4 cases red spots, and in 1 case confluent ill-defined red spots in the hilar region. In 1 rabbit the lung surface was red pink spotted. In all the cases the fluid that could be squeezed out of the lung was sparse.

Frothy fluid in the air ways was noted in only 2 cases.

The lung weight exceeded the normal in 4 cases the highest lung weight was 6.16 g per kg body weight (Table 10).

The water content of the lung was higher than normal in 1 case.

most to 2.3 times the original value, and the pressure in the right atrium to 120 mm saline. Autopsy showed no evidence of pulmonary oedema. Marx et al (1960) infused 8 % dextran in isotonic saline solution intravenously in dogs, 1 ml per minute and kg for 1 hour. Pulmonary oedema did not develop.

Goldenberg et al. (1947) showed that haematocrit and total protein in serum decreased markedly after infusion of dextran in rabbits. The concentration of dextran in blood fell to about 25 % of the original value after 24 hours. As the excretion was insignificant (8 %) and no accumulation in the tissues was noted it was considered probable that dextran is rapidly metabolized in rabbits. Howard et al. (1961) observed that dextran was eliminated from plasma almost equally quickly in oliguric as in non-oliguric patients about 15 % was present in the circulating blood after 24 hours. Distribution of dextran to the extracellular space could only partly explain the continued marked decrease of the dextran concentration, and, consequently dextran must be deposited or metabolized. Wasserman et al. (1955) found that the rate at which dextran disappeared from the circulating blood in dogs depended upon its molecular weight. The concentration of dextran in blood and lymph was the same only for the smallest molecules (molecular weight about 10 000). The heavier molecules occurred in higher concentration in blood than in lymph. At infusion of large amounts, there was, however, very little difference in the dextran concentration between blood and lymph even for dextran with very high molecular weights (about 300,000). Space for dextran with a molecular weight of 50,000 and 400 000 respectively was the same. At a molecular weight of 10,000—35 000 dextran was distributed in a space equalling that of inulin.

The dextran solution was kindly placed at our disposal by AB Pharmacia Uppsala Sweden.

At administration of large amounts of dextran, as in the experiments described in this chapter the animals are made anaemic. A short review of the part played by anaemia in causing pulmonary oedema would therefore be appropriate. The problem has been studied by for instance, Eaton (1947) who was able to produce pulmonary oedema, haemorrhages, and hyperaemia in dogs by heavy loss of blood. The pressure in the systemic arteries fell, but that in the pulmonary artery rose. The pulmonary oedema was aggravated by infusion of saline solutions. Földi et al. (1948) showed that there was a correlation between haemoglobin content and capillary filtration. They considered that oedema in anaemic patients would be due to a pathological increase of permeability in the capillary walls. Fowler et al. (1958) found that in dogs which were hypervolaemic after dextran infusion cardiac output increased only if the animals were anaemic as well. Tybjerg Hansen (1962) formed the hypothesis that the red cells would be important vehicles of fluid and take on fluid at their passage through the capillaries.

### *Own investigations*

The experimental series consists of 41 rabbits in 8 % dextran solution in 0.9 % sodium-chloride solution to which 0.24 g of calcium chloride and 0.42 g of potassium chloride per litre had been added, was infused intravenously in uraemic rabbits, in an amount equalling 12 % of the body weight for 1½ hours (13 ml per minute and kg). The dose of 12 % was chosen since preliminary experiments had shown that almost all the rabbits developed pulmonary oedema if an amount as large as 15 % of the body weight was given. 11 rabbits received the infusion within 1 hour, 10 within 24 hours, 10 within

48 hours, and 10 within 72 hours of the ureteral ligation. The animals that did not die spontaneously were killed by bloodletting 4–5 hours after the infusion. This interval of time was chosen because in preliminary experiments it had been found that dyspnoeic animals began to improve a few hours after the infusion, despite increasing uraemia, and that the dyspnoea lessened gradually or disappeared.

It will be seen from Table 11 that 27 out of 41 animals lived until the planned bloodletting whereas 14 died spontaneously after the infusion. Among the latter 14 animals, 2 had been anuric for only a few hours, 4 for 24 hours, 2 for 48 hours, and 6 for 72 hours. In these animals, autopsy was performed within an average period of 19 (5–45) minutes of death.

#### *Symptoms in vivo*

**Appearance and behaviour** The animals became drowsy after the infusion they did not move but sat cowering and did not react to light prodding. Just before being caught, however they managed to hop about a little, with the exception of the severely uraemic ones, some of which were too weak to move. The eyelid-closure reflex was weak. Spasmodic twitchings arose in some cases. Exophthalmus occurred in many animals during the infusion.

The respiratory rate fell during the infusion from an average of 122 (88–180) to 107 (52–196) per minute. With their noses in the air the animals breathed so quietly that the alae nasi

hardly moved. In 8 animals with three criteria of pulmonary oedema and anuria for at least 48 hours, the respiratory rate immediately before bloodletting averaged 128 (100–160) per minute, whereas in 17 without three criteria of pulmonary oedema and, in most cases, slight uraemia the average rate was 84 (48–124) per minute. With one exception, all the animals with three criteria of pulmonary oedema had dyspnoea. Slight dyspnoea was also noted in 6 animals and severe dyspnoea in 1 animal without three criteria of pulmonary oedema.

**E.C.G. and heart rate** The heart rate before and after the dextran infusion averaged 217 (147–300) and 256 (129–340) beats per minute respectively. In 10 animals with three criteria of pulmonary oedema, mostly of slight degree, the average heart rate immediately before the bloodletting was 255 (174–294) beats per minute. In 16 rabbits without three criteria of lung oedema it was 270 (200–294) beats per minute.

The electrocardiograms were before the infusion normal in 31 rabbits. In 16 of them pulmonary oedema developed later. T wave flattening was seen in 5 out of 41 rabbits before the infusion. In 4 of them pulmonary oedema arose later. Five of the 41 rabbits had before the infusion negative T waves or deformed QRS-complexes. In all of them pulmonary oedema developed later. In comparison with electrocardiograms recorded immediately before the infusion, no or slight changes had occurred immediately after the infusion. In 27 animals, deterioration

Table 11 Results after infusion of dextran solution amounting to the equivalent of 12% of the body-weight for 1½ hours in anuric rabbits whose ureters had been tied 0—2 24 48 and 72 hours respectively before the infusion  
L—at least three criteria of lung oedema S—spontaneous death

Lung weight g/g body weight	Wter content to lung	Pulmonary oedema by gross inspection	Frothy fluid in the air-way	Alveolar oedema	Lung bangles	X ray Cardiac enlargement	Pleural fluid ml	Asciies ml	V P % mg per 100 ml	Post infusion survival hours
L 11.32	87.1	+	+	0	+	+	4	69	108	1 S
L 7.02	83.8	+	+	0	+	+	1	62	86	4
L 6.78	82.4	+	0	0	0	0	0	0	00	4
L 4.73	83.8	0	0	0	+	+	4	85	50	5
L 4.03	82.7	0	0	—	0	0	0	44	01	5
L 4.66	81.7	0	0	—	0	+	0	15	55	4
L 4.35	82.8	0	0	0	0	0	0	2	84	4
L 4.27	81.0	0	0	—	0	0	0	04	60	5
L 1.37	81.7	0	0	0	0	0	0	2	02	3 S
L 3.83	81.0	0	0	—	0	0	0	13	43	4
L 3.60	83.3	0	0	—	0	0	12	15	06	4
Mean 5.19	83.0						2	34	70	
L 11.75	87.7	+	+	0	+	+	2	140	102	0.3 S
L 10.46	83.0	+	+	0	+	+	3	226	118	4 S
L 8.15	81.0	+	+	0	+	+	3	212	114	5
L 7.80	81.9	+	+	0	+	+	3	210	03	0.3 S
L 7.70	82.5	+	+	+	+	+	4	180	103	5
L 6.72	81.3	+	0	0	+	+	1	126	100	1 S
L 5.41	82.5	0	0	0	+	+	7	108	120	4
L 5.17	82.2	0	0	0	+	+	1	106	150	5
L 4.26	83.0	0	0	0	0	+	1	135	115	5
L 1.12	82.8	0	0	0	0	+	2	166	111	1
Mean 7.00	83.8						3	172	116	





in 9 and improvement in 1 (4 rabbits died during or shortly after the infusion) Up to the time of bloodletting the electrocardiograms showed further changes in 9 cases After the infusion electrocardiographic abnormalities were seen in 1 out of 11 rabbits with uraemia for a few hours, in 5 out of 10 with uraemia for 24 hours, in 7 out of 9 with uraemia for 48 hours, and in 3 out of 7 with uraemia for 72 hours At the time of bloodletting 11 rabbits had negative T waves or deformation of the QRS-complex one of them was found to have three criteria of pulmonary oedema

The size of the heart was before the infusion normal in 38 out of 41 animals and increased in 3 all the latter three developed pulmonary oedema during or after the infusion After the infusion the heart size was increased in 31 out of 39 animals (2 died during the infusion) among which pulmonary oedema evidenced by at least three criteria arose in 19 The heart was of normal size in 8 animals one of which developed slight lung oedema It will be seen from Table 11 that immediately before the bloodletting 10 animals had moderately (++) increased heart size 5 of these had three criteria of pulmonary oedema Out of 15 animals with three criteria of pulmonary oedema which were examined before death all had cardiac enlargement Among 14 animals which died spontaneously 5 were X rayed before the respiratory standstill and had enlarged hearts, 8 were X rayed within 5 minutes of the respiratory standstill and of these 2 had enlarged hearts and 4 nor

mal sized hearts, and 3 were not X rayed (more than 5 minutes had elapsed after the respiratory standstill)

X ray of the lungs was normal in all the 41 animals before the infusion In 21 pulmonary changes were noted at the end of the infusion 5 of those which died spontaneously before the planned bloodletting showed changes coded as +++ 2 which died spontaneously and 4 bled animals had ++ and 10 bled animals had + In 2 of the last mentioned animals the degree increased to ++ before the bloodletting

It will be seen from Table 11 that immediately before the bloodletting or in the agonal state 23 animals (56%) had pulmonary changes and that X ray showed no abnormalities in 15 (37%) 3 (7%) were not examined as more than 5 minutes had elapsed after the respiratory standstill Five out of 23 animals with radiographic pulmonary changes had not a sufficient number of other criteria for the diagnosis of pulmonary oedema Three of these 5 had large haemorrhages whereas in 2 no changes were seen in the lungs at autopsy after bloodletting (hyperaemia?) Two rabbits with at least 3 criteria of pulmonary oedema had normal lungs on X ray immediately before the bloodletting both had numerous small haemorrhages macroscopically alveolar oedema microscopically and their lung weights were slightly above the upper limit of normal range 1 rabbit had frothy fluid in the bronchi

### Postmortem findings

Gross observation at autopsy showed normal lung surface in 1 rabbit and haemorrhages in 40. In the form of a few to numerous red dots or spots within a limited area or over the entire surface of the lung. The most severe changes were seen in animals with high-degree uremia. In 20 rabbits the lung surface had the spotted or mottled appearance typical of pulmonary oedema ("+++ or ++") while in 6 the pulmonary changes were assessed as mild (+). Two animals with slight macroscopical pulmonary changes, numerous small red and white dots or red spots, had not a sufficient number of other criteria of pulmonary oedema. In 1 rabbit the lung surface was pink and smooth but pulmonary oedema was present according to four other criteria.

Frothy fluid in the airways was found in 20 rabbits. In 3 of these frothy fluid flowed from the nose and mouth. Four animals with slight pulmonary oedema by at least three criteria had no frothy fluid in the airways.

The lung weights were in 24 out of 41 increased (Table 11) the increase was explained by pulmonary oedema, haemorrhage and/or in spontaneously dead animals, by hyperaemia. One animal had slight pulmonary oedema by three criteria (X ray and gross findings, and water content) but normal lung weight. In 1 the lung weight was below the upper limit of normal range or 6.78 g per kg body weight and numerous diffuse red spots were seen but microscopical examination show

ed haemorrhages without alveolar oedema there was no frothy fluid. X ray showed no abnormalities, and the water content was normal—haemorrhage.

The water content of the lungs was increased in 15 out of 41 animals (Table 11) 11 rabbits with pulmonary oedema evidenced by at least three criteria had normal water contents. In 2 rabbits with slightly increased water contents, a sufficient number of criteria for the diagnosis were not satisfied.

In spite of high lung weights, profuse amounts of fluid macroscopically (often bloodstained, however) on the section surface, and microscopically very protein-poor oedematous fluid, the water content seldom reached very high values. This suggests that haemorrhages and hyperaemia were responsible for a considerable part of the increase in lung weight.

Microscopical examination showed alveolar oedema in 10 cases. In 14 cases of pulmonary oedema evidenced by at least three criteria, there was no alveolar oedema. The absence of visible alveolar oedema may be explained by protein poor oedema fluid with low stainability which has earlier been described by for instance, Vlachar et al., or the explanation may be that alveolar oedema was not present. Arguments against the latter assumption are that oedema fluid flowed profusely from the section surfaces of the lungs and that no alveolar oedema was seen in 9 animals which had pulmonary oedema by all other criteria. The bronchioles showed no changes. In the

arteries there was no periarterial—interstitial oedema.

### Criteria of pulmonary oedema

The six criteria of pulmonary oedema used here agree less well in this than in the other series. The diagnosis of pulmonary oedema was established in 24 cases and in 8 % it was supported by all the six criteria. In 54 % by five, in 25 % by four and in 13 % by three criteria (Radiographs were missing in 3 cases). Among these 24 cases alveolar oedema could not be demonstrated in 58 % and the water content was normal in 37 %. However only 2 out of the 24 animals with pulmonary oedema by at least three criteria had neither alveolar oedema nor a water content above the upper limit of normal range.

In 17 cases the number of positive criteria was less than three, and pulmonary oedema was therefore not considered to be present. Two of these had no positive criteria, 12 had one criterion, and 3 had two criteria of pulmonary oedema.

It will be seen from Diagram 18 and Table 11 that pulmonary oedema following dextran infusion in uraemic rabbits is characterized by marked macroscopical changes, among which haemorrhage predominates, high lung weights in most cases clear X ray evidence of pulmonary changes and frothy fluid in the airways but also by a moderately or slightly increased water content and no or insignificant alveolar oedema.

The great diagnostic difficulties in this series were attached to the differ-

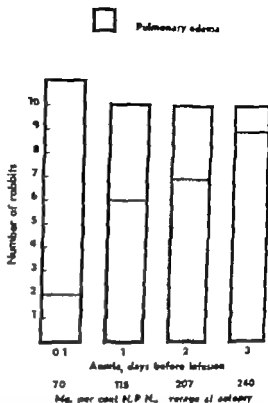


Diagram 7 Occurrence of pulmonary oedema after infusion of dextran solution in amounts equalling 12 % of the body weight for 1½ hours in rabbits which had been anuric for 0.1, 2, 2, and 3 days, respectively before the infusion.

entiation between pulmonary oedema and pulmonary haemorrhage. Bleeding of varying degrees was noted in 98 % of all the rabbits, and all those that had pulmonary oedema by at least three criteria had also haemorrhages into the lung parenchyma. The absence of alveolar oedema may possibly be explained by a low protein content in the oedema fluid the low water content may be attributable to profuse amounts of red cells and possibly dextran in the lungs. This series shows that several methods must be used in evaluation of the oedema state,

so as to ensure a reasonably accurate calculation of the frequency of pulmonary oedema. Yet, despite the use of six methods of examination, the diagnosis of pulmonary oedema is uncertain in the borderline cases.

### *Pulmonary oedema and anaemia*

It will be seen from Table 11 and Diagram 7 that, according to the definition used here, pulmonary oedema arose after dextran infusion in 2 out of 11 rabbits with anaemia for less than 10 hours, in 6 out of 10 with anaemia for 24 hours, in 7 out of 10 with anaemia for 48 hours, and in 9 out of 10 with anaemia for 72 hours. The incidence of pulmonary oedema rose significantly with increasing anaemia. After infusion of dextran in anaemic rabbits the incidence of pulmonary oedema was higher than after transfusion of the same amount of blood  $10 \text{ D}_{50} < p < 0.10$

### *Increase of the extracellular fluid*

#### *Infusion of saline*

Reviews of the literature on pulmonary oedema have been presented by several authors (e.g. 117, 163, 46, 247).

No *in vivo* estimation has been made into the effect of infusion of saline solution on man. Pulmonary oedema 1 man after infusion has been described (6—21, 22, 23, 24 and others) Calligan et al. (1933) and *in vitro* infusion of 400—1500 ml of saline found that the haematocrit and serum protein values fell and that the blood volume increased slightly. With hypertonic solution the increase was somewhat greater than with isotonic solutions. The said disorders lasted 2 hours (most, Doyle et al. (1931) after saline

infusion, observed increased blood volume and raised pressure in the lung capillaries but no disproportionately raised pressure in the pulmonary circulation as compared with the systemic circulation.

The effect of infusion of saline solution in animals with good renal function has been studied by many authors. Cohnheim & Lieblheim (1877) produced pulmonary oedema in rabbits, dogs, sheep, goats, and horses by rapid infusion of large amounts of 0.8% sodium-chloride solution intravenously. Pulmonary oedema, as seen at gross examination, arose in only a small number of the animals, however. It was mild in rabbits, although the infusions were continued until the animals died. Rabbits tolerated an amount of up to 45% of the body weight infused for 2 hours (about 4 ml per minute and kg). Deane & Loye (1888) found that intravenous infusion of at least 3 ml per minute and kg was required to produce pulmonary oedema in rabbits, the total amount being less important. Knoll (1893) also infused 0.6% saline at a rate of 3 ml per minute and kg body-weight and found that a few rabbits did not die until an amount higher than the body weight had been given. Tracheotomized animals tolerated larger amounts than did non-tracheotomized animals, which was thought to be explained by the fact that profuse nasal discharge made breathing difficult in non-tracheotomized animals. Warthen (1906) was able to produce pulmonary oedema in dogs but only by intravenous injection for 25 minutes, at a rate of 10 ml per minute and kg. Labada & Sarnoff (1936) found that pulmonary oedema was more readily produced by infusion into the carotid artery than by infusion into the femoral artery or a vein.

Cohnheim & Lieblheim (1877) noted that acute infusion caused an insignificant rise in blood-pressure and a marked fall of the protein content in the blood-serum. The flow of lymph from the thoracic duct increased 23-fold. The increase originated almost entirely from the abdominal or

arteries there was no periarterial—in terstitial oedema

### *Criteria of pulmonary oedema*

The six criteria of pulmonary oedema used here agree less well in this than in the other series. The diagnosis of pulmonary oedema was established in 24 cases and in 8 % it was supported by all the six criteria in 54 % by five in 25 % by four and in 13 % by three criteria. (Radiographs were missing in 3 cases.) Among these 24 cases alveolar oedema could not be demonstrated in 58 % and the water content was normal in 37 %. However only 2 out of the 24 animals with pulmonary oedema by at least three criteria had neither alveolar oedema nor a water content above the upper limit of normal range.

In 17 cases the number of positive criteria was less than three, and pulmonary oedema was therefore not considered to be present. Two of these had no positive criteria, 12 had one criterion, and 3 had two criteria of pulmonary oedema.

It will be seen from Diagram 18 and Table 11 that pulmonary oedema following dextran infusion in uraemic rabbits is characterized by marked macroscopical changes, among which haemorrhage predominates, high lung weights in most cases clear X ray evidence of pulmonary changes, and frothy fluid in the air ways but also by a moderately or slightly increased water content and no or insignificant alveolar oedema.

The great diagnostic difficulties in this series were attached to the differ

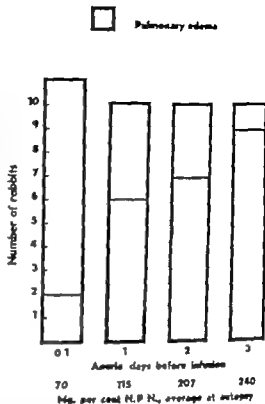


Diagram 7 Occurrence of pulmonary oedema after infusion of dextran solution in animals equaling 12 % of the body weight for 1½ hours in rabbits which had been anaemic for 0, 1, 2, and 3 days, respectively before the infusion.

entiation between pulmonary oedema and pulmonary haemorrhage. Bleeding of varying degrees was noted in 98 % of all the rabbits, and all those that had pulmonary oedema by at least three criteria had also haemorrhages into the lung parenchyma. The absence of alveolar oedema may possibly be explained by a low protein content in the oedema fluid, the low water content may be attributable to profuse amounts of red cells and possibly dextran in the lungs. This series shows that several methods must be used in evaluation of the oedema state.

so as to ensure a reasonably accurate calculation of the frequency of pulmonary oedema. Yet, despite the use of six methods of examination, the diagnosis of pulmonary oedema is uncertain in the borderline cases.

### *Pulmonary oedema and uraemia*

It will be seen from Table 11 and Diagram 7 that, according to the definition used here, pulmonary oedema arose after dextran infusion in 2 out of 11 rabbits with anuria for less than 10 hours, in 6 out of 10 with anuria for 24 hours, in 7 out of 10 with anuria for 48 hours, and in 9 out of 10 with anuria for 72 hours. The incidence of pulmonary oedema rose significantly with increasing uraemia. After infusion of dextran in uraemic rabbits the incidence of pulmonary oedema was higher than after transfusion of the same amount of blood ( $0.05 < p < 0.10$ ).

### *Increase of the extracellular fluid*

#### *Infusion of saline*

Reviews of the literature on pulmonary oedema have been presented by several others (e.g. 117, 166, 236, 247).

Many investigations have been made into the effect of infusion of saline solution in man. Pulmonary oedema in man after infusion has been described (8—21, 91, 103, 136, 194 and others) Gilligan *et al.* (1938) using infusion of 500—1800 ml of saline found that the haematocrit and serum-protein values fell and that the blood haem increased slightly. With hypertonic solutions the increase was somewhat greater than with isotonic solutions. The said disorders lasted for 2 hours (most Doyle *et al.* (1951) after saline

infusion, observed increased blood volume and raised pressure in the lung capillaries but no disproportionately raised pressure in the pulmonary circulation as compared with the systemic circulation.

The effect of infusion of saline solution in animals with good renal function has been studied by many authors. Coburn & Lichtheim (1877) produced pulmonary oedema in rabbits, dogs, sheep, goats, and horses by rapid infusion of large amounts of 0.6% sodium-chloride solution intravenously. Pulmonary oedema, as seen at gross examination, arose in only a small number of the animals, however; it was mildest in rabbits. Although the infusions were continued until the animal died. Rabbits tolerated an amount of up to 48% of the body weight infused for 2 hours (about 4 ml per minute and kg). Dastre & Loye (1888) found that intravenous infusion of at least 3 ml per minute and kg was required to produce pulmonary oedema in rabbits, the total amount being less important. Knoll (1893) also infused 0.6% saline at a rate of 3 ml per minute and kg body weight and found that a few rabbits did not die until an amount higher than the body-weight had been given. Tracheotomized animals tolerated larger amounts than did non-tracheotomized animals, which was thought to be explained by the fact that profuse nasal discharge made breathing difficult in non-tracheotomized animals. Warthen (1935) was able to produce pulmonary oedema in dogs but only by intravenous injection for 34 minutes, at a rate of 10 ml per minute and kg. Lohrke & Sarpoff (1948) found that pulmonary oedema was more readily produced by infusion into the carotid artery than by infusion into the femoral artery or a vein.

Coburn & Lichtheim (1877) noted that saline infusion caused an insignificant rise in blood-pressure and marked fall of the protein content in the blood-serum. The flow of lymph from the thoracic duct increased 25-fold. The increase originated almost entirely from the abdominal or

gans, the lymph flow from the extremities and the head being insignificantly increased. Autopsy showed large amounts of ascitic fluid but rarely pleural effusion. In most animals gross examination of the lungs showed only oedema in the hilar region. Weed & McKibben (1919) noted that the cerebrospinal pressure in cats changed very little during infusion of Ringer's solution. Lands et al. (1940) showed that the greater part of infused saline was retained in the extracellular space. Yeomans et al. (1943) found that the pressure in the pulmonary and the portal veins of dogs rose more markedly than the systemic pressure at infusion of large amounts of saline intravenously. In one animal the pressure in the pulmonary veins rose to 44 cm H<sub>2</sub>O, the heart also increased and the serum protein concentration fell to half during infusion for 30 minutes. Physical signs of pulmonary oedema did not appear. In animals with physical signs of pulmonary oedema (rales heard over the lung fields) the pressure in the jugular vein was not higher than that in animals without pulmonary oedema. Lulsada & Sarnoff (1946) in infusion experiments with saline in dogs, observed a rapidly transient rise of arterial and venous pressure and heart rate. Electrocardiographic examination showed flat or inverted T waves. Opdyke et al. (1948) found that infusion of blood or saline in dogs increased the pressure in the left atrium more than that in the right. Fladdy et al. (1950) noted raised pressure in the pulmonary veins of dogs with pulmonary oedema, according to gross observation at autopsy after infusion of large amounts of saline. Harrison & Liebow (1954) came to the conclusion that infusion in dogs produced pulmonary oedema in those animals which had the highest fluid retention, the largest blood volume and the highest pulmonary vein pressure.

The following authors have studied the effect of fluid infusion under varying experimental conditions.

Kross (1913) showed that intravenous

infusion of saline gave rise to frothy pulmonary oedema in vagotomized rabbits (see the chapter on vagotomy).

Reichsman (1946) demonstrated that pulmonary oedema could be produced regularly in rats which were breathing against inspiratory resistance when they were given saline by rapid intravenous infusion.

Courtice & Korner (1952) produced pulmonary oedema graded by lung weight, in hypoxic rabbits by infusion of Ringer-Locke's solution. Hypoxia (11 % O<sub>2</sub> in the inhaled air) or infusion 160 ml per kg used each by itself did not produce pulmonary oedema.

Wiggers (1939) introduced intravenously 15 litres of saline into a medium-sized dog during the course of 3 hours without signs of developing pulmonary oedema. If however pulmonary pressures were increased still further by means of epinephrine, oedema readily supervened. Korner (1953) produced pulmonary oedema recorded macroscopically and by lung weight, in animals which received both noradrenaline and infusion of saline. When given separately neither the noradrenaline nor the infusion in the doses used gave rise to pulmonary oedema.

Daniel & Cate (1948) found that "wet lung" that is, findings of large amounts of fluid in the lungs at autopsy occasionally occurred in dogs whose chests had been traumatized experimentally. If saline about 200 ml per kg. was given in addition to the traumatization, "wet lung" was invariably produced.

Alwall et al. studied rabbits whose ureters had been tied bilaterally (see Chapter 7).

Alwall, Lindqvist et al. (1963) gave saline intravenously 25 % of the body weight, for 1 1/2 hours (2.8 ml per minute and kg) to rabbits immediately after bilateral ligation of their ureters. Within 48 hours slight radiographic pulmonary changes of "fluid lung" type (+) were seen in 60 % of the cases, slight gross changes as in pulmonary oedema in 0 % and increased

water content (30%) but no frothy fluid was found in the air ways, the lung-weight was normal, and no alveolar oedema was seen. No rabbit had three positive criteria of pulmonary oedema. On the other hand, profuse pleural effusion, a cragging 25 (16-41) ml, and atelectasis were noted.

### *Own investigations*

The experimental series comprised 65 rabbits whose weights ranged between 2.20 and 2.49 kg. At various times after ureteral ligation the animals were given isotonic salt solution intravenously 15% of the body weight for 1½ hours. The purpose was to find out whether different degrees of uraemia would play a part in causing pulmonary oedema. This series forms the basis for the experiments that will be reported in the subsequent chapters and in which additional oedema-producing factors were used.

Another 10 anuric rabbits were given infusions of saline, 50-100% of the body weight. Experiments with such a degree of overhydration do not seem to have been reported earlier.

### *Moderate overhydration (Ringer's solution in amounts equalling 15% of the body-weight)*

Both ureters of 65 rabbits were tied. After intervals of varying length, Ringer's solution in amounts equalling 15% of the body weight was infused for 1½ hours (17 ml per minute and 4 g) Ringer's solution contains 0.0 g of sodium-chloride and 0.24 g of calcium-chloride per 1000 ml. The rabbits were killed by bloodletting 2-4 or 24 hours after the infusion. X-ray and electrocardiographic examinations were made and respiratory rate was measured before the ligation of the ureters, be-

fore the infusion, and before the bloodletting, and, in rabbits that were to be killed after 24 hours, immediately after the infusion as well. The amount of solution for infusion, 15% of the body-weight, was chosen to enable us to make comparison with earlier studies (Alwall, Linderquist, and others) and because it had been found that amounts of 25% produced profuse pleural effusion, ascites, and atelectasis which made the assessment of the chest radiographs difficult and also complicated the experimental conditions in other respects (Alwall & Lindqvist).

The series was divided into 6 groups of 10-11 rabbits each, on the basis of duration of anaemia and overhydration. a) Rabbits receiving the infusion ½ hour, 24 hours, 48 hours, or 72 hours after the ligation of the ureters and bled 2-4 hours after the infusion. b) Rabbits receiving the infusion 48 or 72 hours after the ligation of the ureters and bled after another 24 hours.

**Appearance and behaviour** The animals were not markedly affected by the infusion. Most of them became a little sluggish and sat still for a few hours after the infusion, but then they behaved just like other uraemic rabbits. In most of the highly uraemic rabbits the infusion led to general improvement and prolonged survival times. Among 10 overhydrated rabbits which at the time of infusion had been anuric for 72 hours, all survived another 24 hours, whereas only 16 out of 49 non-overhydrated rabbits which had been anuric for 72 hours before infusion survived a further 24 hours (31%).

The respiratory rate (Table 14) fell in most of the slightly uraemic rabbits after infusion of saline but remained unchanged in rabbits with severe urae-



*mia* One rabbit with pulmonary oedema had a respiratory rate of 84 per minute and dyspnoea. The highest rates, over 120 per minute, were recorded before bloodletting in 3 severely uraemic animals, including 1 with large amounts of pleural fluid and 2 with numerous haemorrhages in the lungs at autopsy and in 4 slightly uraemic rabbits without any pulmonary changes. During the first 24 hours following the infusion, the respiratory rate rose from 88 (70—120) to 105 (66—132) per minute in the group with anuria for 48 hours and fell from 83 (62—110) to 81 (62—102) per minute in the group with anuria for 72 hours.

**ECG and heart rate** The heart rate showed a slight average rise during the infusion irrespective of the degree of uraemia (Table 14). During the first 24 hours after the infusion, the heart rate rose from 231 (200—269) to 232 (200—300) beats per minute in the group with anuria for 48 hours and fell from 263 (202—294) to 252 (178—385) beats per minute in the group with anuria for 72 hours.

Electrocardiographic abnormalities did not occur either before or after the infusion in rabbits with anuria for less than 48 hours. In rabbits with anuria for at least 48 hours the electrocardiographic abnormalities improved during the infusion in 10 cases, became worse in 5 and remained unchanged in the rest. Thus 8 rabbits had before infusion deformed QRS-complexes and/or negative T waves after infusion 4 of these 8 animals had normal electrocardiograms in 1 the T wave

was absent, in 1 negative, and 2 rabbits died during the infusion. In the latter pulmonary oedema was not seen by gross examination at autopsy. Within 24 hours of infusion the number of normal electrocardiograms in the group with anuria for 48 hours decreased from 9 to 8 and the number with absent T waves increased from 1 to 2. In the group with anuria for 72 hours the number of normal electrocardiograms decreased from 6 to 4 and the number of T wave flattening from 3 to 1 whereas that with negative T waves increased from 1 to 4. On one occasion the electrocardiograph was out of order.

**The heart size** Before the infusion 12 % of the rabbits with anuria for at least 48 hours had cardiac enlargement, as against 0 % for those with anuria of shorter duration after infusion (Table 14). The figures were 44 % and 14 % respectively. During the first 24 hours after the infusion the heart size in rabbits with anuria for 48 hours increased in 3 and remained unchanged in 7. In the group with anuria for 72 hours the heart size increased in 2 and remained unchanged in 8 cases. Tables 12 and 13 show the heart size at the time of bloodletting.

Chest radiography was before the infusion normal in all the rabbits excluding 1. This rabbit had ventricular fibrillation in the electrocardiogram, cardiac enlargement (++) and slight pulmonary changes (+) before the infusion, but improved during the infusion the electrocardiographic evidence of ventricular fibrillation disappeared but the pulmonary changes and the

cardiac enlargement persisted (lung weight 5.67 g). After the infusion 5 rabbits with anuria for 48 hours and 1 rabbit with anuria for 72 hours had slight pulmonary changes (+). In 1 rabbit with anuria for 48 hours which lived for another 24 hours, the pulmonary changes disappeared. At the bloodletting slight changes were noted in 3 rabbits (Tables 12 and 13). Of these 5 animals, 1 had several other signs of slight pulmonary oedema, 2 had profuse pleural effusion and atelectasis, 1 had atelectasis without pleural effusion, and in 1 no pulmonary changes were seen at autopsy but large amounts of ascitic fluid were found, and X-ray showed cardiac enlargement.

Besides the afore-mentioned 2 animals that died during the infusion, one more died a few hours before the planned bloodletting and was found to have profuse pleural effusion and atelectatic lungs. The other 62 rabbits were killed by bloodletting (Tables 12 and 13).

Gross observation at autopsy 57 rabbits had pink and 5 had small bluish purple lungs. In 24 animals (including 18 which survived 24 hours after the infusion) the lung surfaces, notably at the base, showed numerous red dots, or brown spots no larger than confetti. Such dots or spots, marked by + in Tables 12 and 13, occurred abundantly in 4 animals of the groups with anuria for 48 and 72 hours. Rabbits which were bled 2 to 4 hours after the infusion and had had anuria for less than 72 hours exhibited no changes.

Tables 12 and 13 show that the average amount of ascitic fluid was greater in overhydrated than in non-overhydrated animals. It increased with increasing uraemia and increasing length of post infusion survival. The individual variations were great however.

Frothy fluid in the air ways 1 rabbit had frothy fluid in the trachea without any other signs of pulmonary oedema.

The lung weight per kg body weight in the 62 bled rabbits was  $4.39 \pm 0.49$  g that is, largely the same as that in normal animals and controls (Table 7). It will be seen from Tables 12 and 13 that there was no difference in lung weight at different degrees of uraemia and different times of bloodletting.

The water content of the lungs. It will be seen from Table 7 that the water content of the lungs of all the 62 bled rabbits was increased the mean being  $82.2 \pm 1.2\%$  that is, higher than in normal and control animals. The degree of uraemia made no difference (Tables 12 and 13). For rabbits bled 2—4 hours after the infusion, the average water content was slightly higher than for those bled 24 hours after the infusion.

Microscopical examination of the spots seen on the lung-surfaces revealed haemorrhages which in many cases were not quite fresh. In other respects the microscopical picture was virtually normal.

#### *Pulmonary oedema and uraemia*

Tables 12 and 13 show that pulmonary

Table 12 Results after infusion of Ringer's solution amounting to the equivalent of 15% of the body weight for 1 1/2 hours in anuric rabbits whose ureters had been tied 0—1 24 48 and 72 hours respectively before the infusion. The animals were bled within 2 to 4 hours of the infusion. *L*—at least three criteria of lung oedema.

Ligature of ureters 0—1 hours after	Lung weight g/kg body weight	Water content of lung %	Pulmonary oedema by gross blood	Frothy fluid in the l. way	Alveolar oedema	Lung changes	X ray	Cardiac enlargement	Pleural fluid ml	Aspirates ml	N.P.V. mg per 100 ml
I. Infusion 0—1 1/2 hours	5.58	80.4	0	0	0	—	—	—	3	25	51
	4.00	81.4	0	0	0	0	0	+	2	2	
	4.78	81.2	—	0	0	0	0	0	4	0	
	4.60	82.4	0	0	—	0	0	0	0	19	
	4.42	83.3	0	0	—	0	0	0	4	20	
	4.42	81.8	0	0	0	0	0	0	1	20	
	4.38	81.6	0	0	0	0	0	0	2	16	
	4.01	82.0	0	0	—	0	0	0	2	5	
	3.93	82.4	0	0	—	0	0	0	1	10	
	2.70	81.6	0	0	0	0	0	0	6	2	
	3.35	82.9	0	0	—	0	0	0	8	28	
	4.38	82.0	0	0	—	0	0	0	4	13	
	5.07	80.4	0	0	—	0	0	0	7	6	
	1.24	81.4	0	0	—	0	0	0	2	37	
	1.82	81.0	0	+	—	0	0	0	4	150	
	4.71	83.0	0	0	—	0	0	+	4	15	
	4.07	80.8	0	0	0	0	0	0	4	250	
II. Infusion 1 1/2 hours	4.00	81.5	0	0	0	0	0	0	2	13	75
	4.16	82.2	0	0	0	0	0	+	2	13	
	4.11	83.4	0	0	0	0	0	0	2	180	
	4.32	82.0	0	0	—	0	0	0	4	10	
	1.19	83.3	0	0	—	0	0	0	0	16	
	1.03	81.8	0	0	—	0	0	0	5	12	
									2	60	

Mean	III. Infusion 15 hours after ligation of nerves	IV Infusion 72 hours after ligation of nerves
147	147	147
150	150	150
153	153	153
156	156	156
159	159	159
162	162	162
165	165	165
168	168	168
171	171	171
174	174	174
177	177	177
180	180	180
183	183	183
186	186	186
189	189	189
192	192	192
195	195	195
198	198	198
201	201	201
204	204	204
207	207	207
210	210	210
213	213	213
216	216	216
219	219	219
222	222	222
225	225	225
228	228	228
231	231	231
234	234	234
237	237	237
240	240	240
243	243	243
246	246	246
249	249	249
252	252	252
255	255	255
258	258	258
261	261	261
264	264	264
267	267	267
270	270	270
273	273	273
276	276	276
279	279	279
282	282	282
285	285	285
288	288	288
291	291	291
294	294	294
297	297	297
300	300	300
303	303	303
306	306	306
309	309	309
312	312	312
315	315	315
318	318	318
321	321	321
324	324	324
327	327	327
330	330	330
333	333	333
336	336	336
339	339	339
342	342	342
345	345	345
348	348	348
351	351	351
354	354	354
357	357	357
360	360	360
363	363	363
366	366	366
369	369	369
372	372	372
375	375	375
378	378	378
381	381	381
384	384	384
387	387	387
390	390	390
393	393	393
396	396	396
399	399	399
402	402	402
405	405	405
408	408	408
411	411	411
414	414	414
417	417	417
420	420	420
423	423	423
426	426	426
429	429	429
432	432	432
435	435	435
438	438	438
441	441	441
444	444	444
447	447	447
450	450	450
453	453	453
456	456	456
459	459	459
462	462	462
465	465	465
468	468	468
471	471	471
474	474	474
477	477	477
480	480	480
483	483	483
486	486	486
489	489	489
492	492	492
495	495	495
498	498	498
501	501	501

Table 13 Results after infusion of Ringer's solution amounting to the equivalent of 15% of the body weight for 1 1/2 hours in anuric rabbits whose ureters had been tied 11 and 3 days respectively before the infusion The animals were bled within 24 hours of the infusion

Infusion time	Weight of rat	Wt. content of gross blood	Wt. content of gross blood	Pulmonary oedema by gross blood flow	Frashy fluid	Alcohol oedema	Lung	X-ray	Cavell enlargement	Pleural fluid	Axilla	Protein	Serum protein
hr	g	%	g	g	ml	ml	g	mm	mm	ml	ml	mg	mg
1.00	1.00	80.5	0	+	0	0	0	+	+	8	102	187	0.7
4.00	4.00	81.0	0	+	0	0	0	+	+	6	92	283	8.8
4.8	4.8	80.0	0	0	0	0	0	+	+	7	185	102	8.5
4.77	4.77	81.8	0	0	0	0	0	+	+	5	82	246	8.6
4.71	4.71	81.2	0	0	0	0	0	+	+	30	85	250	8.9
4.20	4.20	82.0	0	0	0	0	+	+	+	50	206	227	8.1
4.17	4.17	80.5	0	0	0	0	0	+	+	23	130	210	8.0
4.14	4.14	81.0	+	+	0	0	0	+	+	7	73	—	8.0
3.87	3.87	81.0	+	+	0	0	0	0	0	7	30	204	8.0
3.60	3.60	81.7	0	0	0	0	0	0	0	4	250	150	8.0
4.10	4.10	82.1	0	0	0	0	0	0	0	14.7	115	217	8.0
4.03	4.03	81.8	0	0	0	0	0	0	0	8	35	258	8.0
4.18	4.18	70.8	0	0	0	0	0	0	0	13	580	288	8.0
4.23	4.23	81.3	+	+	0	0	0	+	+	0	56	287	8.0
4.21	4.21	82.1	+	+	0	0	0	+	+	4	237	233	8.0
4.20	4.20	82.0	0	0	0	0	0	+	+	0	78	310	8.0
1.13	1.13	80.3	0	0	0	0	0	0	0	31	803	240	8.0
1.10	1.10	80.1	0	0	0	0	0	0	0	16	110	248	8.0
3.03	3.03	80.4	+	+	0	0	0	0	0	0	378	230	8.0
3.81	3.81	81.1	0	0	0	0	0	+	+	6	23	208	8.0
3.7	3.7	82.1	0	0	0	0	0	+	+	8	35	240	8.0
4.16	4.16	81.6	0	0	0	0	0	+	+	10	213	250	8.0

Table 14 *Respiratory rate, heart rate and cardiac enlargement before and after infusion of Ringer's solution 15% of the body-weight for 1 hour in anuric rabbits whose ureters had been tied 0—1, 24, 48 and 72 hours respectively before the infusion*

Number of rabbits	Duration of anuria			
	3 hours 31	24 hours 10	48 hours 21	72 hours 28
Respiratory rate per minute				
before infusion	100 (70—201)	143 (85—300)	103 (50—160)	75 (40—140)
after infusion	90 (43—206)	75 (29—110)	74 (37—120)	76 (48—110)
Heart-rate per minute				
before infusion	311 (156—331)	320 (191—380)	312 (117—383)	235 (178—300)
after infusion	250 (184—285)	324 (130—295)	221 (151—294)	217 (178—300)
Cardiac enlargement				
before infusion	0	0	3	2
after infusion	1	2	12	6

oedema, as defined here, arose after infusion of Ringer's solution in 1 of 10 rabbits which had had uraemia for 72 hours and were killed by bloodletting within 2—4 hours of the infusion. Pulmonary oedema did not develop in the groups that were overhydrated for 1, 24 or 48 hours and bled within 2—4 hours of infusion, nor in the groups that were overhydrated for 24 or 72 hours after infusion and bled 24 hours later. No tendency to storage of fluid in the lungs at high-degree uraemia was noted.

#### Criteria of pulmonary oedema

The animal with pulmonary oedema had slight radiographic pulmonary changes, slight macroscopical pulmonary changes, slightly increased lung weight and relatively greatly increased water content in the lungs. Among the rest of the 62 rabbits, 8 had increased water content, 7 had slight macroscopical changes, 4 had slight

radiographic pulmonary changes, 2 had increased lung weight, 1 had frothy fluid in the air ways, but none had alveolar oedema.

*Severe overhydration (Ringer's solution equalling 50—100% of the body-weight)*

Experiments were carried out with slow infusion of very large amounts of fluid in highly uraemic rabbits.

Five rabbits, anuric for 2 days, were given infusion of Ringer's solution intravenously 50% of the body weight, for 7 hours (1.2 ml per minute and kg). In 1 rabbit with negative T wave before infusion pulmonary oedema developed during the infusion. Three rabbits died post-mortally 7, 8, and 23 hours after the completion of infusion, and 1 rabbit was bled 24 hours after infusion. These 4 rabbits had no pulmonary oedema but their lungs were telestatic. X-ray examination of the lungs showed marked indurations, the water content was increased in only 1 rabbit, averaging 82.9 (79.7—85.7) %; none had frothy fluid in the air ways, the lung-

weights were normal, the average being 5.51 (4.80—6.58) g per kg body weight. Gross observation and microscopical examination showed no signs of oedema. The 4 rabbits had profuse pleural effusion 53 (30—102) ml and massive ascites, 394 (74—902) ml but little pericardial fluid.

Five uraemic rabbits, anuric for 3 days, were given Ringer's solution subcutaneously for 9 hours, 90—100 % of the body weight (1.7 ml per minute and kg). One rabbit died towards the end of the infusion, the others died 1, 6, 8 and 27 hours after completed infusion. None developed pulmonary oedema. X-ray of the lungs showed marked indurations. Autopsy immediately after death showed no frothy fluid in the airways, small dark red lungs, normal (3 cases) or moderately increased (2 cases) lung weight, the average being 6.23 (5.10—7.70) g per kg body weight, normal water content in 4 cases and increased in 1 case, average 83.3 (81.4—88.5) %. Little pleural fluid 6 (1—16) ml and pericardial fluid and large amounts of ascitic fluid 500 (132—815) ml. All the tissues seemed to be imbued with fluid. Microscopically atelectasis without oedema was seen.

The series was not completed as no pulmonary oedema but only atelectasis developed. These experiments showed that heavy overhydration of severely uraemic rabbits did not produce pulmonary oedema if the animal survived the infusion.

## Infusion of mannitol solution

Mannitol has been used for assessment of the extracellular space in the body (62, 130, 188, 233, 234). Mannitol does not penetrate the red blood-cells and is not bound to protein. In man it has a smaller space (about 16 % of the body weight) than have chloride and sodium (about 23 % and 25 % respectively). Mulrow et al. (1956) found that nephrectomized dogs had a mannitol space of 22 % which after 30 hours had risen to 48 %. Last et al. (1952)

observed that after infusion of mannitol intravenously 8 hours were required for the mannitol concentration in ascitic fluid and interstitial fluid to become equally high.

How large a part of mannitol is broken down has not been made clear. Dominguez (1947) found that in man 20 % is broken down and 80 % is excreted in the urine in 24 hours. According to Smith et al. (1940), 15 % and according to Schwarz et al. (1950) about 10 % is broken down in man. Elkington (1949) found that the breakdown of mannitol in anuric patients is insignificant. Houch (1951) in dogs, observed no destruction of mannitol, since in 11 hours all of an injected quantity can be recovered in the urine. Carr et al. (1933, 1938) noted a slight rise of blood sugar in rabbits after oral administration of mannitol.

Side-effects of mannitol used clinically have occurred in the form of dyspnoea and pulmonary oedema in patients with cardiac failure (36), lower urinary-tract diseases (178) and renal insufficiency (21). Symptoms resembling those of acute water intoxication have also been described (223 a).

Barry & Berman (1961) observed that the blood volume of an anuric patient increased greatly during infusion of 100 ml of 20 % mannitol.

Marx et al. (1960) gave dogs intravenous infusions of fluid in amounts equalling 10 % of the body weight for 1 or 2 hours. One series received a solution of 5 % mannitol and 5 % sorbitol, and another series was given 5 % glucose. In a third series, a solution of 6 % dextran in 0.9 sodium chloride was infused for 1 hour in an amount corresponding to 5 % of the body weight *in vivo*. The blood volume increased with mannitol/sorbitol to the same extent as with glucose but not as much as with dextran. Pulmonary oedema was not noted.

When given in large doses to rats, mannitol depresses cerebral function, just like other alcohols (Macht & Ting 1932).

### *Own investigations*

To find out the frequency of pulmonary oedema in uraemic rabbits at an increase of the extracellular fluid, 5.5 % mannitol solution was infused intravenously in amounts equalling 15 % of the body weight for 5 hours.

The experimental conditions originally planned were the same as those in the saline experiments. Preliminary trials showed, however, that such a technique could not be used, because 3 animals which received infusions of 5.5 % mannitol solution intravenously 15 % of the body weight for 1 1/2 hours, became severely ill during or immediately after the infusion. Their breathing became slow and irregular and their eyes large. They died suddenly, one of them after a drawn-out cry. Pulmonary oedema was not seen at autopsy. In this series, bloodletting was planned to take place 24 hours after the infusion. In the experiments with saline, it will be seen from the following account that the survival time was shorter in many cases. Bloodletting was therefore carried out when the animals had passed into a clearly moribund state.

In 20 rabbits the ureters were tied bilaterally. Two days later when the N.P.N. level was around 180 mg per 100 ml, they were given infusions of isotonic (5.5 %) mannitol solution to which had been added 0.24 g of calcium chloride and 0.42 g of potassium chloride per litre of fluid. The infused amount corresponded to 15 % of the body weight immediately before the infusion. The infusion time was 5 hours.

Among the 20 rabbits in the series, 1 died during the infusion, 1 immediately after the end of it, 6 within 12 hours,

and 9 within 12 to 24 hours of the infusion. Three survived 24 hours after the infusion.

The last mentioned 3 rabbits were bled 24 hours after the infusion. In all 10 rabbits were bled within 16 (15—24) hours of the infusion and 10 died spontaneously. Of the latter only 5 could be examined promptly after death, while their bodies were still warm and soft in the other 5 cases rigor mortis had already set in at autopsy.

**Appearance and behaviour.** The animals were sluggish, trembling, and weak. Most of the time they lay flat on their abdomens, fore legs wide apart. They did not salivate and did not have exophthalmus. The corneal reflexes and nose-reflexes were normal. Dyspnoea rarely occurred. Most of the animals died quietly without convulsions.

The respiratory rate fell in the 19 rabbits that survived after the infusion, on an average from 124 (76—176) before to 73 (56—133) per minute after infusion, and then remained unchanged until death. Only 1 animal, which had profuse pleural effusion (53 ml) was dyspnoeic.

**E.C.G. and heart-rate.** The heart rate rose in 19 rabbits, on an average from 226 (164—294) beats per minute before infusion to 257 (168—327) beats per minute after infusion. Immediately before the bloodletting the heart rate in 10 rabbits averaged 187 (88—308) beats per minute.

The electrocardiogram was normal before infusion in 17 out of 20 animals. In 2 the T wave was isoelectric and in 1 negative. Immediately after



the infusion 7 out of 19 surviving animals had normal electrocardiograms, 4 had isoelectric T waves 7 had negative T waves, and 1 had a deformed QRS-complex. Immediately before the bloodletting 2 out of 10 surviving animals had normal electrocardiograms 5 had negative T waves and 3 had deformed QRS-complexes the electrocardiographic abnormalities had not disappeared in any of the animals since the infusion. Among those 11 that lived longest, 3 had normal electrocardiograms and 1 had isoelectric T wave immediately after the infusion.

The heart size assessed in the radiograph increased during the infusion in 9 of 19 animals (one died during infusion). The enlargement persisted or increased in 5 which were examined immediately before the bloodletting. The size of the heart was not influenced by the infusion but remained normal until bloodletting in 4 animals which survived at least 18 hours after the infusion.

X ray evidence of slight pulmonary changes was noted in 2 of 19 rabbits by examination immediately after the infusion. It will be seen from Table 15 that immediately before the bloodletting pulmonary changes were present in 5 animals. Three of the latter had atelectasis resulting from profuse pleural effusion (40—62 ml).

Serum electrolyte concentrations after death. The serum potassium concentration at the bloodletting averaged 7.4 (5.8—8.5) mEq per litre (6 animals) and the serum sodium concentration 103 (95—115) mEq per litre (5 animals).

Gross observation at autopsy. Gross evidence of slight pulmonary oedema was seen in only 1 bled rabbit (Table 15). No such evidence was found in those that died spontaneously.

The amount of pleural fluid averaged 18 (4—62) ml and that of ascitic fluid 100 (8—250) ml the subcutis was very rich in fluid.

Frothy fluid in the air ways was not found in any animals.

The lung weight was raised in 3 bled rabbits. The average lung weight of the bled animals was 4.80 (4.0—10) g of the spontaneously dead non-rigid animals 4.80 (3.80—6.30) g and of the spontaneously dead rigid ones 6.98 (6.20—8.12) g per kg body weight. In comparison with the control series the differences were insignificant.

The water content of the lungs was slightly raised in 2 animals. The average water content for the bled animals was 82.4 (80.7—84.0) % which was somewhat higher than for the control series.

Microscopical examination showed slight alveolar oedema in 1 rabbit. Minor haemorrhages were seen in 4. In all the animals the alveolar septa were a little thicker than normally.

#### *Frequency of pulmonary oedema*

Pulmonary oedema as defined here occurred in 1 of 10 bled animals but in none of the non rigid spontaneously dead ones. As regards those examined after rigor mortis had set in the occurrence of mild degrees of pulmonary oedema cannot be assessed with certainty but oedema of high degree was definitely not present.



the infusion 7 out of 19 surviving animals had normal electrocardiograms 4 had isoelectric T waves 7 had negative T waves and 1 had a deformed QRS complex. Immediately before the bloodletting 11 out of 10 surviving animals had normal electrocardiograms, 5 had negative T waves and 3 had deformed QRS-complexes the electrocardiographic abnormalities had not disappeared in any of the animals since the infusion. Among those 5 that lived longest 3 had normal electrocardiograms and 1 had isoelectric T wave immediately after the infusion.

The heart size assessed in the radiograph increased during the infusion in 9 of 19 animals (one died during infusion) The enlargement persisted or increased in 5 which were examined immediately before the bloodletting The size of the heart was not influenced by the infusion but remained normal until bloodletting in 4 animals which survived at least 18 hours after the infusion.

X ray evidence of slight pulmonary changes was noted in 2 of 19 rabbits by examination immediately after the infusion It will be seen from Table 15 that immediately before the bloodletting pulmonary changes were present in 5 animals. Three of the latter had atelectasis resulting from profuse pleural effusion (40—62 ml)

Serum-electrolyte concentrations after death The serum potassium concentration at the bloodletting averaged 7.4 (5.8—8.5) mEq per litre (6 animals) and the serum sodium concentration 103 (95—115) mEq per litre (5 animals)

Gross observation at autopsy Gross evidence of slight pulmonary oedema was seen in only 1 bled rabbit (Table 15) No such evidence was found in those that died spontaneously

The amount of pleural fluid averaged 18 (4—62) ml and that of ascitic fluid 100 (8—250) ml the subcutis was very rich in fluid.

Frothy fluid in the air ways was not found in any animals

The lung weight was raised in 2 bled rabbits. The average lung weight of the bled animals was 4.86 (4.0—11.10) g of the spontaneously dead non rigid animals 4.89 (3.80—6.30) g and of the spontaneously dead rigid ones 6.98 (6.20—8.12) g per kg body weight. In comparison with the control series the differences were insignificant

The water content of the lungs was slightly raised in 2 animals. The average water content for the bled animals was 82.4 (80.7—84.0) % which was somewhat higher than for the control series

Microscopical examination showed slight alveolar oedema in 1 rabbit Minor haemorrhages were seen in 4 In all the animals the alveolar septa were a little thicker than normally

#### *Frequency of pulmonary oedema*

Pulmonary oedema as defined here occurred in 1 of 10 bled animals but in none of the non rigid spontaneously dead ones. As regards those examined after rigor mortis had set in the occurrence of mild degrees of pulmonary oedema cannot be assessed with certainty but oedema of high degree was definitely not present

blood sugar concentration immediately after the infusion averaged 0.79 (0.72—0.87) g per 100 ml.

After the infusion, the animals first improved temporarily and then became increasingly sluggish, mostly lying motionless flat on their abdomens, seemingly exhausted, and walking with an unsteady gait. Nyctalagmus was noted in many cases. Muscular twitchings occurred in different extremities or muscle groups. The saliva flowed. Their condition was as a rule worst 6—8 hours after the infusion. The blood-sugar concentration of 9 cases averaged 0.19 (0.11—0.33) g per 100 ml about 6 hours after the infusion whereas in 1 it was still 0.70 g per 100 ml 6 hours after the infusion. Nine rabbits were bled at this stage, as their general condition was very poor (Table 16). At the bloodletting the serum contained, on an average, 105 (89—113) mEq of sodium and 0.9 (5.6—7.9) mEq of potassium per litre.

From 8 hours after infusion up to the bloodletting 24 hours after infusion many of the animals improved. Most of them were still very weak, however, and 2 died spontaneously during this period. At the bloodletting 24 hours after the infusion (10 rabbits) N.P.V. averaged 204 mg per 100 ml (Table 10). The serum sodium was 111 (98—120) and serum potassium 0.7 (5.2—7.7) mEq per litre (8 and 5 rabbits, respectively). The blood-sugar was normal in 2 animals.

The respiratory rate fell during the infusion, from an average of 126 (72—138) to an average of 9 (28—120) per minute. In 9 moribund dogs, about 6

hours after infusion, the respiratory rate averaged 69 (20—110) per minute at the bloodletting, and in 10 animals 24 hours after infusion it averaged 78 (51—118) per minute.

E.C.G. and heart rate. The heart rate changed on an average very little during and after the infusion.

Electrocardiography showed slightly flattened T waves, but only 1 rabbit had negative T wave after the infusion. About 6 hours after the infusion 9 moribund rabbits had abnormal electrocardiograms, showing isoelectric T waves in 3 negative T waves in 4 and serious deformation of the QRS-complexes in 2. Among 10 rabbits which survived until the bloodletting 24 hours after the infusion the electrocardiograms were still normal in 8, whereas 2 had negative T waves.

The heart size increased during the infusion to ++ in 3 rabbits and to + in 7 whereas it remained unchanged in 10. The examination was not successful in 1 rabbit. At the bloodletting 2 animals had cardiac enlargement, in 3 which had cardiac enlargement immediately after the infusion, the heart was of normal size.

Slight radiographic pulmonary changes were present in 1 of 23 rabbits after the infusion and persisted at the bloodletting 24 hours later. No pulmonary changes were noted in the other rabbits. At autopsy 45 ml of pleural fluid were found in 1 rabbit although X ray immediately before death had not shown any abnormalities, mistake?

Gross observation at autopsy. All the tissues (subcutis, viscera etc.) appear

### *Criteria of pulmonary oedema*

The rabbit with "pulmonary oedema" had all the other criteria but lacked frothy fluid in the air ways. With the exception of positive findings as regards chest X ray in 4 and lung weight and water content in 1 all the criteria were negative in the other 9 bled animals

### *Increase of all the fluid spaces*

#### *Infusion of glucose solution*

Cohnheim & Lichtheim (1877) induced pulmonary oedema in dogs and rabbits by infusing very large amounts of 3 % glucose solutions intravenously. Fremont Smith & Forbes (1927) showed that intravenous infusion of hypotonic glucose solutions in animals caused a marked rise of the intracranial pressure. Warthen (1935) did not note pulmonary oedema after intravenous infusion averaging as much as 134 ml of 5 % glucose solution per kg of body weight for only 24 minutes. The blood-sugar rose, on an average from 124 mg to 454 mg per 100 ml. Infusion of 10 % solution caused fatal cerebral oedema in 30 % of the animals within a few hours. Yeomans et al. (1943) did not note pulmonary oedema after infusion of 150-300 ml of 5 % glucose solution per kg body weight and hour. Lands et al. (1940) found that the larger part of infused 5 % glucose solution accumulated in the extracellular space. All the experiments quoted here were made on animals with intact renal function.

In 1911 glucose infusions were introduced by Matas in human therapy. Altshuler et al. and Gilligan et al. (1938) noted a rapidly transient fall of haematocrit and serum protein values and an increase of the plasma and blood volumes after intravenous infusion of glucose. Hyper-

tonic glucose solutions increased the blood volume slightly more than did isotonic glucose solutions. Murphy et al. (1941) observed the same increase of the plasma volume and the venous pressure after infusion of 5 % glucose solution and the same amount of saline, respectively.

Funkh Brentano et al. (1939) found that administration of large amounts of saline through a gastric tube causes no symptoms in rabbits but that the same amount of water produces fatal cerebral oedema. They considered that cerebral signs of water intoxication in rabbits are due less to the increase of the total fluid than to the expansion of the intracellular volume.

#### *Own investigations*

This series of experiments were designed to find out whether an increase of the fluid content in the body without a corresponding increase (relative decrease) of the body's salt content would produce pulmonary oedema in uraemic rabbits.

In 28 rabbits both ureters were ligated. Two days later the  $\text{N P N}$  level averaged 180 mg per 100 ml. Isotonic glucose solution (5.5 %) to which had been added 0.24 g of calcium chloride and 0.42 g of potassium chloride in a volume equalling 15 % of the body weight was infused intravenously for 1 1/2 hours.

Appearance and behaviour. During the infusion the rabbits were sluggish *sat still most of the time* and were slow in reacting to stimuli. Marked exophthalmus was noted. Towards the end of the infusion many of them bent their heads back. At times they were restless and had convulsive twitchings. 4 rabbits died with convulsions. Only a few had dyspnoea. In 9 cases the

blood sugar concentration immediately after the infusion averaged 0.79 (0.72—0.87) g per 100 ml.

After the infusion, the animals first improved temporarily and then became increasingly sluggish, mostly lying motionless flat on their abdomens, seemingly exhausted, and walking with an unsteady gait. Nystagmus was noted in many cases. Muscular twitchings occurred in different extremities or muscle groups. The saliva flowed. Their condition was as a rule worst 6—8 hours after the infusion. The blood-sugar concentration of 9 cases averaged 0.19 (0.11—0.23) g per 100 ml about 6 hours after the infusion, whereas in 1 it was still 0.70 g per 100 ml 6 hours after the infusion. Nine rabbits were bled at this stage, as their general condition was very poor (Table 16). At the bloodletting the serum contained, on an average, 105 (89—113) mEq of sodium and 6.9 (4.6—7.9) mEq of potassium per litre.

From 8 hours after infusion up to the bloodletting 24 hours after infusion many of the animals improved. Most of them were still very weak, however, and died spontaneously during this period. At the bloodletting 24 hours after the infusion (10 rabbits) V.P.N. averaged 204 mg per 100 ml (Table 16). The serum sodium was 111 (98—120) and serum-potassium 6.7 (5.2—7.7) mEq per litre (8 and 5 rabbits, respectively). The blood-sugar was normal in 2 animals.

The respiratory rate fell during the infusion, from an average of 126 (72—138) to an average of 70 (28—120) per minute. In 9 moribund dogs, about 6

hours after infusion the respiratory rate averaged 69 (20—110) per minute at the bloodletting and in 10 animals 24 hours after infusion it averaged 78 (51—118) per minute.

**E.C.G. and heart rate** The heart rate changed on an average very little during and after the infusion.

Electrocardiography showed slightly flattened T waves, but only 1 rabbit had negative T wave after the infusion. About 6 hours after the infusion 9 moribund rabbits had abnormal electrocardiograms, showing isoelectric T waves in 3, negative T waves in 4, and serious deformation of the QRS-complexes in 2. Among 10 rabbits which survived until the bloodletting 24 hours after the infusion, the electrocardiograms were still normal in 8, whereas 2 had negative T waves.

The heart size increased during the infusion to ++ in 3 rabbits and to + in 7, whereas it remained unchanged in 13. The examination was not successful in 1 rabbit. At the bloodletting 2 animals had cardiac enlargement in 3 which had cardiac enlargement immediately after the infusion the heart was of normal size.

Slight radiographic pulmonary changes were present in 1 of 23 rabbits after the infusion and persisted at the bloodletting 24 hours later. No pulmonary changes were noted in the other rabbits. At autopsy 45 ml of pleural fluid were found in 1 rabbit, although X-ray immediately before death had not shown any abnormalities. mistake?

**Gross observation at autopsy** All the tissues (subcutis viscera etc.) appear

ed to be very rich in fluid. The brain was not examined.

Only 1 out of 4 rabbits that died in connection with the infusion had signs of pulmonary oedema at autopsy namely a white-spotted (+) lung surface a lung weight of 7.75 g per kg body weight, and a water content in the lungs of 84.5% frothy fluid in the air ways was not seen.

Five rabbits, which died spontaneously 1–23 hours after the infusion, had large amounts of pleural fluid. In 1 animal autopsy immediately after death showed no evidence of pulmonary oedema the lung weight was 4.18 g per kg. The other rabbits could not be examined until rigor mortis had occurred. In 3 of them pulmonary oedema was not seen by gross observation and in 1 the picture could not be assessed.

One out of 19 bled animals had macroscopical evidence of slight pulmonary oedema (Table 16) in the form of numerous red dots and white spots. Five rabbits had, in addition, a few small haemorrhages and 4 had bluish collapsed lungs whereas 9 had normal lung surfaces.

Frothy fluid in the air ways was not seen in any animal.

The lung weight in the bled animals was increased in 1 out of 19 rabbits (Table 16) this animal had slight hyperaemia and minor haemorrhages established microscopically. The average lung weight was 4.46 (3.74–5.87) g per kg body weight which is only slightly higher than in the control series.

The water content of the lungs was

slightly increased in 4 out of the 19 bled rabbits in Table 16. The averaged content was 82.3 (80.1–84.0)%, which is somewhat higher than in the control series.

Microscopical examination showed alveolar oedema in only 1 rabbit. Haemorrhage and atelectasis were seen in rabbits with macroscopically observed dots and bluish lungs, respectively.

#### *Frequency of pulmonary oedema*

A sufficient number of criteria of pulmonary oedema as defined here were not found in any rabbit after the infusion.

#### *Criteria of pulmonary oedema*

Among the 19 bled animals, 4 had an increased water content in the lungs. Radiographic pulmonary changes occurred in 1 macroscopical and microscopical evidence of pulmonary oedema was noted in 1 each and increased lung weight in 1. Frothy fluid in the air ways was not present in any animal.

#### *Infusion of urea solution*

Urea is distributed in a space approximately to that of the total fluid (201, 203, 235) and equilibrium between the concentrations in different organs is reached within an hour. Rolfs (1943) however found that the concentration of urea is higher in red cells than in plasma.

Vanquellin & Segales (1927) seem to have been the first to give urea to animals. Stretcher (1928) injected 200 ml of 10–20% urea intravenously in dogs with normal renal function. The concentration of urea in the blood rose maximally to 700

Table 18. *R. ratt.* *at* infusion / 5.5% glucose solution amounting to the equivalent / 15% / the body-weight /  $r = 1$  / hours in anesthetic rabbit whose arteries had been tied 3 d before the infusion. The rabbits were killed by bloodletting.

Lung- right g./g. body- right	$\text{H}_2\text{O}$ content 1 lung %	Tracheal arteries by gross observation	Tracheal fluid in the arteries	Alcohol	Lung changes	X-ray	Cardiac enlargement	Internal fluid ml	Active ml	M. P. X. mg per 100 ml	Post- infusion arterial hours
5.87	81.0	0	0	0	-	-	-	4	64	150	5
5.08	82.5	0	0	-	0	0	0	3	23	115	6
4.53	81.6	0	0	+	0	0	0	1	116	101	6
4.81	84.7	0	0	0	0	0	0	2	129	175	21
4.74	83.1	0	0	-	0	0	0	0	305	277	21
4.71	83.4	0	0	0	+	+	+	5	32	106	24
4.53	82.8	0	0	0	0	0	0	6	0	104	25
4.58	83.6	+	0	0	-	-	-	3	33	193	0
4.31	82.8	0	0	0	0	0	0	0	37	161	0
4.22	82.5	0	0	0	-	-	-	25	53	142	7
4.26	82.7	0	0	0	0	0	0	4	237	174	24
4.21	81.8	0	0	-	0	0	0	2	120	192	24
4.11	81.7	0	0	0	0	0	+	0	15	198	6
4.10	84.0	0	0	-	-	-	-	11	63	174	6
4.08	83.0	0	0	-	-	-	-	14	112	196	24
4.01	82.5	0	0	0	-	-	-	4	28	170	6
4.06	82.0	0	0	-	0	0	0	2	300	197	24
3.83	81.3	0	0	-	0	0	0	0	19	256	24
3.74	80.4	0	0	-	0	0	0	45	224	192	24
Mean	81.3	0	0	0	0	0	0	8	109	181	16



mg per 100 ml Vomiting occurred at 200 mg dyspnoea and restlessness at 300 mg tremor after 400 mg convulsions after 500 mg, and diarrhoea unconsciousness, and death after 600 mg per 100 ml No signs of haemolysis were noted. The blood pressure rose markedly Autopsy of the lungs showed hyperaemia but no pulmonary oedema. Similar results were obtained by Grollman & Grollman (1959) in nephrectomized dogs in which the urea concentration was kept constant by peritoneal dialysis. Mayerson et al (1962) observed increased permeability in the lymphatics at high urea levels (at least 8%) Schiano & d Onafrio (1957) found that in rabbits whose ureters had been tied the N.P.N level averaged initially 35 mg, after 24 hours 172 mg and after 52 hours 275 mg per 100 ml Injection of 200 mg of urea per kg body weight (the concentration of the solution is not stated) led to death within a few hours.

Creery (1951) noted marked haemolysis at irrigation of the bladder in man, using 1.8% urea in aqueous solution Roberts (1955) showed that aqueous solutions of urea promptly produced haemolysis both in vivo and in vitro in diluted as well as in concentrated solutions. If 25% urea was added to isotonic solutions of glucose or sodium chloride haemolysis did not occur.

Fremont Smith & Forbes (1927) showed that intravenous injection of 50% urea solution causes a marked fall of the intracranial pressure David & Andersson (1959) found that hypertonic urea solution lowers the intracerebral pressure in bilaterally nephrectomized monkeys Alexander et al (1961) noted a great increase of the plasma volume in dogs after intravenous infusion of 30% urea in 10% invertose solution.

### *Open investigations*

The purpose of this experimental series was to find out whether overhydration and a high concentration of urea by administration of urea solutions, would produce pulmonary oedema in anuric rabbits.

The ureters were tied in 12 rabbits weighing between 2.20 and 2.40 kg. Sterile urea powder was dissolved in a sterile fluid to the desired concentration immediately before the infusion.

The animals in the first series which had been anuric for 48 hours, died during the infusion of isotonic (1.8%) urea solution in water for 1½ hours, in an amount equalling 15% of the body weight The technique was therefore varied as follows Longer infusion time, hypertonic solutions different solvents different lengths of the interval between ureteral ligation and infusion and subcutaneous instead of intravenous injection Irrespective of technique the rabbits became severely ill after the infusion. The desired combination of fluid retention and high urea concentration could be carried through only for a short time

a) Intravenous infusion of 1.8% urea solution in an amount corresponding to 15% of the body weight for 1½ hours in 4 rabbits which had been anuric for 48 hours.

During the infusion signs of restlessness appeared the animals swallowed frequently and their tongues became bluish Staggering and exophthalmus were not noted All the animals died during the infusion in convulsions Autopsy showed no evidence of pulmonary oedema but blood serum was red in colour after centrifugation (haemolysis)

b) Intravenous infusion of 1.8% urea solution in an amount corresponding to 15% of the body weight for 1 hour in 4 rabbits which had been anuric for 48 hours.

The reactions of the animals were the same as in the previous series. 2 rabbits died during the infusion, 1 died 3 hours later whereas 1 survived 24 hours, alert but slightly trembling, and with an N.P.N. level of 344 mg per 100 ml. The latter was killed by bloodletting. The rabbit and the one that died spontaneously after 3 hours had no pulmonary oedema. Autopsy after 1 1/2 hours of the two that died during the infusion showed slight increase of the weight and water content of the lungs but no frothy fluid in the air ways. One of them had slight radiographic pulmonary changes immediately before death and the other had slight macroscopical signs of pulmonary oedema.

c) Subcutaneous infusion of 1.8 % urea solution in an amount equalling 15 % of the body weight. The volume was divided into five doses which were infused at one-hour intervals. The series comprised 10 rabbits which had been anuric for 48 hours.

One rabbit died spontaneously during the infusion and 6 died within 2 to 18 hours of the infusion. 3 rabbits, which were all 18-24 hours after the infusion, were killed by bloodletting. The clinical picture after infusion was similar in all the animals. They showed lack of strength, trembled and twitched but reacted to stimuli. The saliva flowed from their mouths, they lay with their heads resting on the floor between their paws. Nycturia and exophthalmus were not noted. Death occurred unnoticeably without convulsions, dyspnoea, etc. The respiratory rate fell, on an average from 142 to 110 per minute during the infusion. Finally breathing became increasingly lower and weaker. 6 of the 9 rabbits that survived the infusion had deformed QRS-complexes or negative T waves afterwards, and in 2 of the rest changes developed later. All those that died postmortal were examined and autopsied. 4 of these cases autopsy

was performed within 15 minutes and in the other 3 within 60 to 80 minutes of death. 2 of the latter had the highest lung weights in the series. At autopsy N.P.N. averaged 334 (302-396) mg per 100 ml. As will be seen in Table 17 all the criteria of pulmonary oedema were lacking.

(d) Subcutaneous infusion of 1.8 % urea solution in amounts equalling 15 % of the body weight. The volume was divided into five doses which were infused at one-hour intervals. The series comprised 4 rabbits which had been anuric for 1 hour.

All the 4 animals remained lively during and after the infusion up to the bloodletting 24 hours later. E.C.G. remained normal. N.P.N. at the bloodletting averaged 280 (264-294) mg per 100 ml. All the criteria of pulmonary oedema were lacking. The lung weight averaged 4.26 (4.08-4.43) g, the water content 82.4 (82.1-82.8) %, the pleural fluid 0.3 (0-1) ml, and the ascitic fluid 5 (0-16) ml. The lungs looked quite normal.

(e) Subcutaneous and intravenous infusion, respectively of 1.8 % urea in 5.5 % glucose solution in amounts equalling 15 % of the body weight for 4 hours. Each series comprised 2 animals which had been anuric for 48 hours.

Exophthalmus, irregular breathing, staggering gait, and weakness were noted in all the animals, most markedly in the intravenous-infusion group. All had severe electrocardiographic changes at the end of the infusion. The rabbits in the intravenous-infusion group died shortly after the infusion and those in the subcutaneous-infusion group within about 11 hours after the infusion. X ray and autopsy showed evidence of pulmonary oedema.

Table 17 Results after infusion of 1.8% urea solution subcutaneously 15% of the body-weight for 4 hours in anuric rabbits whose ureters had been tied 2 days before the infusion S = spontaneous death

Lung weight g/100 body weight	Water content lung %	Pulmonary oedema by gross observation	Protein fold in the way	Al oedem	Lo g	X-ray	Cardiac large-ment	Pleural fluid ml	Ascorbic ml	N. P. N mg per 100 ml	Post-infusion survival hours
6.37	80.1	0	0	—	—	—	—	0	0	—	0
0.07	82.3	0	0	—	—	—	—	0	0	344	5
5.81	82.6	0	0	—	—	—	—	4	2	325	7
5.57	80.7	0	0	—	—	—	—	0	0	326	4
5.14	80.8	0	0	0	—	—	—	0	45	379	18
5.08	83.1	0	0	—	0	0	0	0	2	302	2
1.92	81.8	0	0	—	0	0	+	5	75	370	20
1.92	81.2	0	0	0	0	0	0	0	20	382	10
4.01	80.8	0	0	—	0	0	+	0	11	352	7
5.28	81.5	0	0	0	0	0	0	8	253	306	24
Mean								2	59	354	

(f) Subcutaneous or intravenous infusion of 1.8 % urea in 10 % invertose solution in amounts equalling 10—15 % of the body weight for 1—3 hours. The series comprised 12 rabbits which had been anuric for 48 hours.

(1) 6 rabbits received a volume of 15 % of their body weights intravenously for 3 hours

All the animals became very poor lying still, flat on their abdomens nystagmus was noted in many cases, dyspnoea in none. E.C.G. showed severe changes. The animals died quietly during or shortly after the infusion, excluding one which survived 6 hours.

(2) 3 rabbits received amounts equalling 10 % of their body weights intravenously for 1 hour and 3 the same amounts subcutaneously

2 were killed by bloodletting after 24 hours, and 4 died spontaneously after 2—12 hours of increasing weakness. In the intravenous-infusion group E.C.G. showed severe changes the animals in the subcutaneous-infusion group had no E.C.G. changes and they did not survive longer. Autopsies showed none of the criteria of pulmonary oedema.

(g) Intravenous infusion of 30 % urea in 10 % invertose solution in amounts equalling 1.8 % of the body weight for 30 minutes. The series comprises 4 rabbits which had been anuric for 1 hour

1 animal had trembling after the infusion, the others were unaffected. E.C.G. changes appeared immediately after the infusion. 3 rabbit died spontaneously

within about 20 hours. 2 were bled after 24 hours, one of them in a very poor general condition. N.P.N. averaged 350 (362—390) mg per 100 ml. Autopsies showed none of the criteria of pulmonary oedema.

(h) In 2 rabbits extremely severe pulmonary oedema developed after infusion of 20 ml of 50 % urea intravenously for 2—4 minutes.

### *Toxicity of urea*

Infusion of isotonic urea solution in amounts equalling 10—15 % of the body weight had a toxic effect on moderately uraemic rabbits, whether the solvent was water 5.5 % glucose, or 10 % invertose, and even with slow subcutaneous infusion. Rabbits with slight uraemia, on the other hand, tolerated slow subcutaneous infusion of the same amount of isotonic urea solution. Anuric rabbits died at N.P.N. levels of 300—400 mg per 100 ml even when they were not overhydrated and the rise in N.P.N. was largely attributable to urea.

### *Frequency of pulmonary oedema*

Pulmonary oedema as defined here occurred in 2 animals which were given 50 % urea solution by rapid intravenous infusion but did not occur in animals which were given isotonic urea solution or 30 % urea solution for 30 minutes. A few criteria of pulmonary oedema were present in animals that died during the infusion.

*Survey* See page 146

## Pulmonary oedema following anaesthesia induced with barbiturates

Moon & Morgan (1936) noted pulmonary oedema in dogs which had received repeated large doses of phenobarbitone intravenously for several days. Autopsies revealed petechial haemorrhages on the lung surfaces and the lungs were filled with blood and oedematous areas of bronchopneumonia were also seen. The oedema was considered to be a result of increased permeability in the lung capillaries caused by prolonged shock. Gorth (1945) reported pulmonary oedema of 3 rabbits in connection with vagal apnoea, which was produced under deep narcotical narcosis (sodium isopropyl B bromallyl N methyl malonylcarbamide) Alton et al. (1949) had technical difficulties at dialysis of rabbits by means of the artificial kidney because many of the animals, which were treated under Narcotical anaesthesia, died from pulmonary oedema. An analysis of the experimental conditions showed that the anaesthesia was the cause of the complicating lung oedema. Stone & Loew (1949) found that adrenalin produced severe pulmonary oedema in rabbits which were pretreated with phenobarbitone. Barriety & Kohler (1953) and Kohler & Barbe (1954) described pulmonary oedema in rabbits following intravenous injection of the barbiturate compound 'Somnifène'. Pulmonary oedema arose only in those animals which received such large doses that they died. Curare ganglion blocking agents, and, to a smaller extent, atropine increased the tendency to pulmonary oedema after barbiturates.

Durlacher et al. (1950) found that post

mortal lung oedema could be induced with Nembutal® (sodium ethyl-1-methylbutyl malonylcarbamide) (see Chapter 4)

Experiments have also been reported in which barbiturates were capable of protecting animals against pulmonary oedema.

Brann (1953) induced pulmonary oedema in vagotomized rabbits by rapid intravenous infusion of saline solution. Pre-treatment with 0.1 g of phenobarbitone per kg body weight prevented the development of pulmonary oedema. Lüscher & Sarnoff (1946) noted a similar effect of phenobarbitone in experiments on dogs. Poulsen (1946) found that aprobarbitone protected mice against lung oedema from inhalation of  $CO_2$ .

Under the experimental conditions applied in this investigation the animals developed anoxia and a survey of the literature on the subject will therefore be of interest.

Naum (1951) on the basis of the relevant literature and his own experiments concluded that acute anoxia increases capillary permeability in extremely severe cases only and that there is no evidence that a long standing mild hypoxia would increase the susceptibility to oedema. Courtice & Horner (1952) were however able to induce pulmonary oedema in hypoxic rabbits by infusion of Ringer Locke solution.

Each by itself neither hypoxia nor infusion produced pulmonary oedema. Pattie (1936) exposed one lung of an animal to anoxia while the other one was normally oxygenated. After pulmonary oedema had been induced in the animal (with adrenal anaemia, phosgene) he could not demonstrate greater susceptibility to oedema on the anoxic side. Poulsen (1936) was not able to find pulmonary oedema in mice unless the oxygen concentration in the inspired air was as low as 5%. Poulsen (1935, 1936) noted a low frequency of pulmonary oedema in mice which had inspired 10–15% CO<sub>2</sub> for 5 minutes and a low frequency of severe pulmonary oedema after inspiration of 25% or higher concentrations. Rabbit also developed pulmonary oedema after inhalation of CO<sub>2</sub>, which potentiates the anaesthetic action of barbiturates.

The following authors deal with some other technical problems which are of interest here.

Gordh (1935) found that the depth of narcosis induced by 'Narkotol' in rabbit can be graded according to the recording in tetraoal paralysis. He also showed that lowering of the head end could prevent pneumonia after administration of doses that caused pneumonia when the head end was slightly raised. The heart was dilated during the narcosis. Dundee & Richards (1937) observed that penthotal-sodium is metabolized lower in narcosis than by normal animals, and that the susceptibility to penthotal-sodium increases in narcotic animals.

#### Narkotol—anaemia—fluid retention lung

To enable a study of the pathogenesis of radiographic pulmonary changes designated as uraemic lung, uraemic pulmonary oedema etc., Alwall, at the end of the 1910s, started experi-

ments on rabbits, designed to provoke slowly developing radiographic pulmonary changes of the afore mentioned type (Alwall 1932, 1954, Alwall & Lundquist 1933, Lundquist 1933, Alwall et al. 1963).

The rabbits ureters were tied under local anaesthesia. Immediately after the operation, Ringer's solution in amount equaling 10, 15, and 25 % of the animal's body weight was infused intravenously for 1–2 hours. The animals were X-rayed daily in the erect and the lateral position for the presence of pulmonary changes and pleural effusion, respectively. In the series of 10 % Ringer's solution no pulmonary changes were noted in the series of 15 % Ringer's solution pulmonary changes occurred in 24 % of the cases, but they were only slight and did not appear until the 2nd postinfusion day later. The frequency was thus too low and the survival time (observation period) for the narcotic animals too short. It was not possible to study as planned, the effect of infiltration of the blood in vivo for the purpose of removing the administered amount of fluid, that is, the effect of dehydration on the pulmonary changes. In the series of 25 % Ringer's solution later on enlarged in co-operation with the present author radiographic pulmonary changes appeared sooner or later in half to two-thirds of the animals, but the film were difficult to assess because of profuse pleural effusion.

In 1919 the experimental technique was therefore modified as follows, on the basis of the observation that 'Narkotol' narcosis produces pulmonary oedema in rabbit. The ureters were tied under 'Narkotol' narcosis; 1.3 ml of 'Narkotol' solution per kg body weight was injected for 20 minutes. The narcosis was of sufficient depth and duration to allow painless ligation of the ureters and exposure of the jugular vein for insertion of a cannula through which Ringer's solution was infused for

# Pulmonary oedema following anaesthesia induced with barbiturates

Moon & Morgan (1936) noted pulmonary oedema in dogs which had received repeated large doses of phenobarbitone intravenously for several days. Autopsies revealed petechial haemorrhages on the lung surfaces and the lungs were filled with blood and oedematous areas of bronchopneumonia were also seen. The oedema was considered to be a result of increased permeability in the lung capillaries caused by prolonged shock. Gordh (1945) reported pulmonary oedema of 3 rabbits in connection with vagal apnoea, which was produced under deep narcotic anaesthesia (sodium isopropyl 3-bromoethyl N-methyl malonylcarbamide). Atwell et al. (1949) had technical difficulties at dialysis of rabbits by means of the artificial kidney because many of the animals, which were treated under Narkotal anaesthesia, died from pulmonary oedema. An analysis of the experimental conditions showed that the anaesthesia was the cause of the complicating lung oedema. Stone & Loew (1949) found that adrenalline produced severe pulmonary oedema in rabbits which were pretreated with phenobarbitone. Barlett & Kohler (1953) and Kohler & Barbe (1954) described pulmonary oedema in rabbits following intravenous injection of the barbiturate compound Somnifène. Pulmonary oedema arose only in those animals which received such large doses that they died. Curare ganglion blocking agents, and, to a smaller extent atropine, increased the tendency to pulmonary oedema after barbiturates.

Durlacher et al. (1950) found that post

mortal lung oedema could be induced with Nembutal® (sodium-ethyl-1-methylbutyl malonylcarbamide) (see Chapter 4).

Experiments have also been reported in which barbiturates were capable of protecting animals against pulmonary oedema.

Braun (1933) induced pulmonary oedema in vagotomized rabbits by rapid intravenous infusion of saline solution. Pretreatment with 0.1 g of phenobarbitone per kg body weight prevented the development of pulmonary oedema. Luisada & Sarnoff (1946) noted a similar effect of phenobarbitone in experiments on dogs. Poulsen (1945) found that aprotobarbitone protected mice against lung oedema from inhalation of CO<sub>2</sub>.

Under the experimental conditions applied in this investigation the animals developed anoxia and a survey of the literature on the subject will therefore be of interest.

Nairn (1951) on the basis of the relevant literature and his own experiment concluded that acute anoxia increases capillary permeability in extremely severe cases only and that there is no evidence that a long standing mild hypoxia would increase the susceptibility to oedema. Courtice & Korner (1952) were however able to induce pulmonary oedema in hypoxic rabbits by infusion of Ringer Locke solution.

injected intravenously into an ear vein, 1.7 ml per kg body weight the animals were weighed immediately before the induction of narcosis. The injection was made as rapidly as was possible without killing the animal. Deep narcosis was desired, with momentary respiratory standstill or a few minutes of bradypnoea (18 respirations per minute or less).

The doses of 'Narkotal' were thus larger than those used by Alwall et al., who gave 1.2 ml per kg body weight in inducing narcosis with abolition of pain for 20 minutes. In these experiments the rate of injection was adjusted to the respiratory rate and deep narcosis was aimed at (18 respirations per minute, or lower preferably without respiratory standstill). The higher dose 1.7 ml, instead of 1.2 ml, per kg body weight was established after pilot experiments using Alwall's et al. technique. (a) 5 rabbits which had been anuric for 24 hours were anaesthetized with 1.2 ml of Narkotal per kg body weight. Ringier solution was then infused in amounts equalling 15 % of the body weight for 1 hour. The animals were bled 1 hour later. In 2 of the cases pulmonary changes (+) were seen in the radiograph, but autopsy showed no evidence of pulmonary oedema. Bronchiolitis did not occur. (b) 10 rabbits which had been anuric for 48 hours were given an average of 1.2 (1.0—1.6) ml of Narkotal per kg body weight and Ringier solution. In 3 of them slight pulmonary oedema developed (as well as radiographic changes) and bronchiolitis occurred in 7.

These experimental conditions were not suitable as a higher frequency of pulmonary changes during an observation period shorter than that used by Alwall et al. was considered desirable. The 'Narkotal' dose was therefore increased to 1.7 ml per kg body weight. Further increase was not tried as it was found that highly

uraemic animals did not tolerate the last mentioned dose (Table 18).

At a fixed induction time and dose of Narkotal highly uraemic animals became very feeble and died during injection at a rate that affected slightly uraemic animals very little. A fairly constant depth of sleep was therefore tried, which, when the dose was the same, meant that the duration of narcosis varied individually. In highly uraemic animals reactions to pain and reflexes gave unsatisfactory guidance as regards the depth of narcosis. The animals could still show reflexes and react to pain after marked bradypnoea or even apnoea had set in. Therefore, the respiratory rate (abdominal respiration) was used as an indicator of the depth of narcosis.

However with this method, too it was found very difficult to control the depth of narcosis as exactly as was desired particularly in the highly uraemic animals. The difference between the amount of anaesthetic that produced a respiratory rate of 16 per minute and that which caused prolonged respiratory standstill was in many cases very small. The respiratory rate did not fall below 20 per minute in 19 % of the cases. In 23 % of the animals artificial respiration with a rocking device was necessary because of prolonged respiratory standstill lasting for 10 (2—50) minutes (Table 18). As a rule, a smaller amount of Narkotal was required to produce the desired bradypnoea in highly uraemic rabbits than in those with slight uraemia. Respiratory standstill was more difficult to avoid in highly uraemic ani-



1 1/2 hours. The anuric animals were given penicillin and streptomycin prophylactically against infectious complications during the uraemic stage. Pulmonary changes designated as "fluid lung" or "fluid retention lung" (Alwall 1963) appeared in 8% of the animals that were operated upon under Narkotal narcosis. In those which after operation under Narkotal narcosis were given Ringer's solution in amounts equalling 15% of the body-weight, radiographic pulmonary changes occurred in one-third within the first 24 hours and in two-thirds during the observation period. In 31 rabbits (weighing 1.11—2.49 kg) whose ureters were tied under Narkotal narcosis, the lung weight at bloodletting 48 hours later averaged 4.10 (3.40—9.86) g per kg body weight. In 36 rabbits (weighing 1.79—2.48 kg) in which ureteral ligation was made under Narkotal narcosis and infusions were given as above, the average lung weight 48 hours later was 4.9 (3.6—6.1) g per kg body weight.

In one experimental series (Lunderquist) the administered amount of fluid was removed by ultrafiltration of the blood *in vivo* about 20 hours after the operation under Narkotal narcosis and infusion of Ringer's solution the radiographic pulmonary changes and pleural effusion that had already appeared disappeared in response to the ultrafiltration treatment and no radiographic pulmonary changes developed subsequently despite increasing uraemia.

The lung weight rose in the animals that exhibited radiographic changes. Bergman summarises the result of the microscopical examination of lungs with radiographic changes as follows: "— was noted pulmonary oedema of varying degree with a protein concentration in the oedematic fluid sufficiently high to be demonstrated by the histological technique used. Inflammatory changes were not noted nor any fibrin deposit of the type

described by Hopps Wissler as an autopsy finding in uraemic patients. This report also includes an experimental series in which amounts of Ringer's solution equalling 15% of the animals' body weights were infused 2 days after preteral ligation under local anaesthesia. Under these experimental conditions, overhydration, which was induced in the presence of uraemia, did not cause any radiographic pulmonary changes during the observation period, that is, before the animals died in uraemia.

Alwall et al. designated the noted radiographic changes in the experimental animals as "Narkotal uraemia fluid-retention lung" concluding that overhydration is an essential pathogenic factor as it is in uraemia fluid retention lung in patients (13, 16, 18, 20, 21).

### *Own investigations*

This series was used for studying the frequency of pulmonary oedema following Narkotal narcosis at varying degrees of uraemia in non-overhydrated and overhydrated rabbits whose ureters had been tied bilaterally.

Narkotal® solution contains iso-propyl beta bromovaleryl-L-malonycarbamide sodium, 0.055 g phenarone, 0.08 g glycerine 0.05 g, *aqua sterilisata ad 1 ml*.

2, 24, 48 or 72 hours after their ureters had been tied bilaterally the animals were anaesthetized with Narkotal 1.7 ml per kg body weight. The following experiment had to be carried out before the technique suitable for the applied conditions could be established.

The rabbits were placed on their back with their heads hanging about 2 cm lower than their hind paws. Narkotal was

Kindly placed at our disposal by AB Astra Sweden.

Table 10. Cardiac enlargement and pulmonary changes seen in the radiographs before Narbotal anaesthesia and after 'Narkotal' anaesthesia and infusion of Ringer's solution, 15% (the body-weight for 1 1/2 hours, in rabbits whose urea had been lifted 2, 24, 48 and 72 hours, respectively before the anaesthesia. The figures denote the number of rabbits in each group

		Duration of anuria Number of animals				Total
		2 hours	24 hours	48 hours	72 hours	
Cardiac enlargement	0	23	9	21	21	74
	+	—	1	3	2	6
	++	—	—	—	1	1
	+++	—	—	—	—	—
	No X-ray	—	—	—	2	2
	0	11	6	11	14	42
	+	11	4	9	9	33
	++	1	—	3	3	7
	+++	—	—	—	—	—
	No X-ray	—	—	1	1	2
Pulmonary changes	0	23	10	22	23	78
	+	—	—	2	1	3
	++	—	—	—	—	—
	+++	—	—	—	—	—
	No X-ray	—	—	—	2	2
	0	23	9	19	18	69
	+	1	1	3	5	10
	++	—	—	1	2	3
	+++	—	—	—	—	—
	No X-ray	—	—	1	1	2
Total		23	10	24	26	83

were subjected to autopsy atelectasis was noted but there was no pulmonary oedema. Microscopical examination was performed in 3 cases: no bronchial oedema was seen.

Appearance and behaviour after narcosis. The rabbits were sluggish and weak for several hours. Most of them then recovered seemingly completely, a few deteriorated further and a few remained weak up to the time of blood letting.

The respiratory rate which before the narcosis averaged 120 (38—168)

per minute was 1 hour after the narcosis 71 (20—144) per minute.

E.C.G. and heart rate. The heart rate before the narcosis averaged 223 (120—300) and 1 hour after the narcosis 240 (182—327) beats per minute.

The electrocardiograms were unchanged after the narcosis in comparison with those taken before the narcosis in all the animals that had had anuria for 2, 24 or 48 hours before the narcosis. Among 12 animals with anuria for 72 hours before the narcosis, the E.C.G. was normal in 11 and the

Table 18 Length of survival time of death and duration of 'Narkotal' anaesthesia and lowest respiratory rate during anaesthesia in those animals that survived the anaesthesia

Time of death	Duration of anuria				Total
	2 hours	21 hours	48 hours	72 hours	
during anaesthesia before infusion	3	2	11	19	35
during infusion	1	0	5	0	6
spontaneous death within 24 hours of infusion	1	0	10	12	23
bled 24 hours after infusion	32	20	26	28	106
Number of anaesthetized rabbits	37	22	52	50	160
Duration of anaesthesia for rabbits that survived the anaesthesia	31 (14—60)	27 (10—55)	40 (12—120)	52 (15—125)	39 (10—125)
Lowest respiratory rate during anaesthesia					
20 per minute	4	7	0	8	25
4—18 per minute	19	9	29	22	79
respiratory standstill (artificial respiration)	11	4	0	10	31

male and was in them more often followed by cardiac arrest in spite of artificial respiration. Animals that became dyspnoeic and cyanotic tolerated only very slow injection of Narkotal solution. The duration of narcosis in rabbits which needed artificial respiration averaged 54 (16—125) minutes and for those in which a satisfactory depth of narcosis was not achieved it lasted 27 (10—75) minutes. Highly uraemic animals took a long time to recover from the narcosis.

At least half the animals in each series were immediately after the completion of the injection of Narkotal given Ringer's solution by intravenous infusion 15% of the body weight for 1 1/2 hours, lying in the supine position during the procedure. Those that after the termination of the experiment survived 24 hours were bled. Four

moribund animals with severe changes were bled within 22 hours of the experiment. Respiratory rate measurements, electrocardiograms, and chest films were taken before ligation of the ureters before induction of narcosis in rabbits with anuria for at least 24 hours, within 1 hour of the experiment and immediately before the bloodletting.

#### Non-overhydrated animals Reaction to 'Narkotal' narcosis

Deaths during 'Narkotal' narcosis. Among 37 animals with anuria for 2 hours 8% died, among 21 animals with anuria for 24 hours 0% died among 52 animals anuric for 48 hours 21% died and among 50 animals with anuria for 72 hours 30% died during the narcosis (Table 18). 20 animals

1 had no pulmonary changes. Dyspnoea was difficult to detect in animals with severe uraemia and a poor general condition.

The respiratory rate in 20 rabbits with pulmonary oedema immediately before the bloodletting averaged 135 (80—190) per minute. Among 25 animals which at the bloodletting had respiratory rates exceeding 120 per minute, 9 had pulmonary oedema, 2 had profuse pleural effusion, 7 had other slight pulmonary changes, and 7 had no or insignificant pulmonary changes.

E.C.G. and heart rate E.C.G. after the infusion showed no abnormalities in 58 animals, absent T waves in 14, negative T waves in 8 and marked deformation of the QRS-complex in 1. Among the 23 rabbits with E.C.G. abnormalities after infusion, pulmonary oedema developed later in 14. Nineteen rabbits with pulmonary oedema had immediately before the bloodletting a mean heart rate of 230 (182—316) beats per minute. E.C.G. showed no abnormalities in 12 cases, absent T waves in 3 and negative T waves in 4. Serum potassium was determined at the bloodletting in 16 highly uraemic animals. Among 11 with serum potassium exceeding 7.3 mEq per litre, the E.C.G. showed no abnormalities in 3, absent T waves in 3, negative T waves in 3, and a deformed QRS-complex in 1. Three rabbits with serum potassium levels of 7.4, 7.7 and 10.2 mEq per litre respectively had pulmonary oedema of moderate degree.

The heart shadow was enlarged in overhydrated rabbit only 13 out of

18 overhydrated rabbits with at least three criteria of pulmonary oedema had cardiac enlargement. 15 out of 41 rabbits with cardiac enlargement had at least three criteria of pulmonary oedema. Among rabbits with amounts of ascitic fluid exceeding 180 ml, 23 out of 25 did not have cardiac enlargement. A calculation of the correlation between the amount of fluid in the pericardial sac and the heart size in 86 overhydrated rabbits showed  $r = 0.31 \pm 0.10$ . The method of measuring the pericardial fluid was not satisfactory however. Electrocardiographic changes before the bloodletting were present predominantly in rabbits with severe uraemia. The correlation between the heart size and the severity of electrocardiographic changes was poor  $r = 0.07 \pm 0.10$  as measured in 82 overhydrated rabbits anaesthetized with "Narkotal".

Cardiac enlargement and slight pulmonary changes were present before the narcosis in 1 rabbit. The animal was bled 11 hours later and was found to have pulmonary oedema. Before the narcosis 8 animals had cardiac enlargement, pulmonary oedema developed in 6 animals.

Following overhydration, 11 rabbits had cardiac enlargement and pulmonary changes. Lung oedema developed in 52 %. 72 rabbits had after the infusion cardiac enlargement without pulmonary changes. Lung oedema developed in 23 %. 45 rabbits had normal heart-size and no pulmonary changes after the infusion. Lung oedema arose in 20 %.

Radiographic pulmonary changes

T wave was absent in 1. One hour after the narcosis these 12 had normal electrocardiograms the T wave was absent in 2 and negative in 4 and the QRS complex markedly deformed in 2. In most cases the E.C.G. abnormalities persisted or became more severe during the first 24 hours following the narcosis.

The heart shadow before the narcosis was enlarged in 1 rabbit. Radiographic pulmonary changes were not noted. One hour after the narcosis chest X ray in the non infusion group (46 animals) showed no abnormalities.

#### *Overhydrated animals*

##### *Reaction to Narkotal' narcosis and overhydration*

Deaths during infusion of Ringer's solution 6 out of 89 animals died during the infusion. Autopsy was performed in 4 cases and revealed bluish red sanguineous lungs but no frothy fluid in the air ways. In 1 case the lungs were pink. histological examination showed only mild atelectasis.

As regards the appearance and behaviour the animals did not differ much from the non-overhydrated series.

The respiratory rate before the narcosis averaged 125 (58—200) and after narcosis and infusion 75 (32—132) per minute.

E.C.G. and heart rate. The heart rate before the narcosis averaged 224 (152—300) and after narcosis and infusion 240 (125—308) beats per minute.

The electrocardiograms were normal both before narcosis and after infusion.

In all the animals in the groups with narcosis plus infusion 2 and 24 hours after ureteral ligation. In the group of 24 rabbits with narcosis plus infusion 48 hours after ureteral ligation the E.C.G. showed no abnormalities in 20. Isoelectric T waves in 2 and negative T waves in 2 after narcosis and infusion the E.C.G. was normal in 15 the T wave isoelectric in 3 and negative in 3 and the QRS-complex was deformed in 1 (the record is missing in 1 case). In the group of 26 rabbits with narcosis plus infusion 72 hours after ureteral ligation the E.C.G. before narcosis showed no abnormalities in 21. Isoelectric T waves in 2 and negative T waves in 2 (the record is missing in 1). After narcosis and infusion the E.C.G. was normal in 10 the T wave isoelectric in 10 and negative in 6.

The heart size before the narcosis was increased in 9 % of the rabbits, as against 48 % after narcosis and infusion (Table 19).

Pulmonary changes were noted before the narcosis in 4 % and after the infusion in 16 % of the animals, predominantly in those with high degrees of uraemia (Table 19).

#### *Summary of the findings in non-overhydrated and overhydrated rabbits with at least three criteria of pulmonary oedema following Narkotal' narcosis*

Appearance and behaviour. Severe dyspnoea was present in 10 animals. 13 of these had pulmonary oedema. 2 had profuse pleural effusion. 3 had bronchitis or marked bronchiolitis and

non-overhydrated animals. In the non-overhydrated group the highest value was 85.8 % one overhydrated rabbit had a water content of 91.1 % which was the highest value recorded for all the series. In spite of very high water contents, even those amounting to 91.1 % and 90.7 % the alveolar oedema was clearly visible microscopically. Most of the animals with more than 200 ml of ascitic fluid had a low water content in the lungs.

Microscopical examination showed alveolar oedema in 30 animals. The preparation was accidentally lost in 1 case no examination was performed in 27 cases, as the macroscopical pictures and the lung weights were normal (Tables 20 and 21). Alveolar oedema was seen in all except 3 of the 29 rabbits that had pulmonary oedema evidenced by at least three criteria. Among the 3 exceptions, 1 had very large haemorrhages, 1 had hyperaemia and 1 had only slight changes. In 4 cases alveolar oedema was noted but pulmonary oedema according to three criteria could not be established. The alveolar oedema was clearly visible in 18 cases in 12 cases it was only seen focally or indistinctly. Most of the rabbits had alveolar oedema with fibrinous stringy exudate, others had haemorrhagic alveolar oedema with protein-rich exudate, and 1 rabbit had thin vacuolized oedematous fluid. In some animal all the different types were seen and the degree of visibility and stringiness varied from one part of the lung to another.

### *Criteria of pulmonary oedema*

The six criteria of pulmonary oedema used here showed relatively good correlation in this series. In 29 cases the diagnosis of pulmonary oedema was established and in 34 % it was supported by all the six criteria in 34 % it was supported by five in 21 % by four and in 10 % by three criteria. Radiographs were missing in 5 cases and the microscopical preparation was lost in 1 case.

In 91 cases the number of positive criteria was less than three and pulmonary oedema was therefore considered not present. 65 of these rabbits had no positive criteria 16 had one criterion, and 10 had two. Radiographs are missing in 2 cases. In 26 cases the lungs were not examined microscopically as the gross pictures and the lung weights were normal and there was no frothy fluid. None of these rabbits had more than one positive criterion of pulmonary oedema.

"Pulmonary oedema following Narkotal" narcosis in uraemic rabbits (Diagram 18 Tables 20 and 21) was characterized by distinct gross pulmonary changes, in most cases without haemorrhages and usually clearly visible alveolar oedema. In the majority of cases X ray and measurements of lung weight to body weight ratio provided clear evidence. Observations on the water content and frothy fluid, on the other hand, were less valuable in estimating the degree of the pulmonary oedema that arose under the experimental conditions of this study.

A characteristic feature of the lung oedema in this series was that, when

were present in 31 out of 120 animals. 7 spontaneously dead animals were not examined. Films of those dying spontaneously were taken only if there was time to do so during agony. The X ray findings were in most cases consistent with other findings. Radiographic changes were present in 10 rabbits without pulmonary oedema by at least three criteria. 6 of these had atelectasis and profuse pleural effusion. 1 had mild pneumonia and 3 had moderate lung bleedings whether they also had hyperaemia is uncertain. 3 rabbits had at least three criteria of pulmonary oedema without radiographic changes. In 2 of these the oedema was located basally and in 1 it appeared here and there in the lungs.

Gross observation at autopsy. 39 rabbits had normal lung surfaces without changes; most of them being slightly uraemic non-overhydrated rabbits. A few red or brown dots and spots were seen on the lung surfaces of 32 rabbits most of which were highly uraemic. Microscopical examination showed focal haemorrhages. 13 overhydrated rabbits with in most cases profuse pleural effusion had bluish red atelectatic lungs. In several rabbits in the two last mentioned groups numerous white dots were seen on the lung surfaces.

Gross evidence of pulmonary oedema was present in 36 animals. 13 of these had slight generalized changes in the form of numerous red dots or slightly diffuse spotty markings and/or localized changes in the form of reddish brown, in most cases numerous,

spots basally or in the hilar region, or spotty markings in a small part of the lung. 11 rabbits had moderate macroscopical changes in the form of numerous large irregular spots, innumerable small red dots, or mottled lung surfaces seen only in the lower lobes or parts thereof. In the 12 rabbits with the most extensive macroscopical changes the lungs had mottled surfaces of varying colour-combinations, for instance, purple—beige blue—brown red—brown—white red—pink. In 29 out of the above-said 36 rabbits the macroscopical diagnosis of pulmonary oedema was further supported by at least two other criteria. All the rabbits with pulmonary oedema by at least three criteria had also gross evidence of pulmonary oedema (Diagram 18).

Frothy fluid in the air ways was found in 23 animals. 1 of these had bronchitis and the rest had pulmonary oedema. 7 animals with at least three criteria of pulmonary oedema had no frothy fluid in the air ways.

The lung weight was increased in 25 animals. In 4 cases it was within normal range although at least three other criteria of pulmonary oedema were present (Tables 20 and 21). In these cases the changes were localized in a small part of the lung.

The water content of the lung was increased in 34 animals. 7 animals with pulmonary oedema had normal water contents. 12 without three criteria of pulmonary oedema had increased water contents (Tables 20 and 21). The average water content of the lung was much higher in overhydrated than in

non-overhydrated animals. In the non overhydrated group the highest value was 83.8 % one overhydrated rabbit had a water content of 91.1 % which was the highest value recorded for all the series. In spite of very high water contents, even those amounting to 91.1 % and 90.7 % the alveolar oedema was clearly visible microscopically. Most of the animals with more than 200 ml of ascitic fluid had a low water content in the lungs.

Microscopical examination showed alveolar oedema in 80 animals. The preparation was accidentally lost in 1 case; no examination was performed in 27 cases, as the macroscopical pictures and the lung weights were normal (Tables 20 and 21). Alveolar oedema was seen in all except 3 of the 20 rabbits that had pulmonary oedema evidenced by at least three criteria. Among the 3 exceptions, 1 had very large haemorrhages, 1 had hyperaemia, and 1 had only slight changes. In 4 cases alveolar oedema was noted but pulmonary oedema, according to three criteria, could not be established. The alveolar oedema was clearly visible in 18 cases; in 12 cases it was only seen focally or indistinctly. Most of the rabbits had alveolar oedema with fibrinous stringy exudate; others had haemorrhagic alveolar oedema with protein-rich exudate, and 1 rabbit had thin vacuolized oedematous fluid. In some animals all the different types were seen and the degree of visibility and stringiness varied from one part of the lung to another.

### *Criteria of pulmonary oedema*

The six criteria of pulmonary oedema used here showed relatively good correlation in this series. In 29 cases the diagnosis of pulmonary oedema was established and in 34 % it was supported by all the six criteria. In 34 % it was supported by five, in 21 % by four and in 10 % by three criteria. Radiographs were missing in 5 cases and the microscopical preparation was lost in 1 case.

In 91 cases the number of positive criteria was less than three and pulmonary oedema was therefore considered not present. 65 of these rabbits had no positive criteria, 18 had one criterion and 10 had two. Radiographs are missing in 2 cases. In 26 cases the lungs were not examined microscopically as the gross pictures and the lung weights were normal and there was no frothy fluid. None of these rabbits had more than one positive criterion of pulmonary oedema.

"Pulmonary oedema following Narkotal narcosis in anaemic rabbits (Diagram 18, Tables 20 and 21) was characterized by distinct gross pulmonary changes, in most cases without haemorrhages and usually clearly visible alveolar oedema. In the majority of cases X-ray and measurements of lung weight to body weight ratio provided clear evidence. Observations on the water content and frothy fluid, on the other hand, were less valuable in estimating the degree of the pulmonary oedema that arose under the experimental conditions of this study.

A characteristic feature of the lung oedema in this series was that, when



were present in 31 out of 120 animals 7 spontaneously dead animals were not examined. Films of those dying spontaneously were taken only if there was time to do so during agony. The X ray findings were in most cases consistent with other findings. Radiographic changes were present in 10 rabbits without pulmonary oedema by at least three criteria. 2 of these had atelectasis and profuse pleural effusion 1 had mild pneumonia and 8 had moderate lung bleedings whether they also had hyperaemia is uncertain 3 rabbits had at least three criteria of pulmonary oedema without radiographic changes. In 2 of these the oedema was located basally and in 1 it appeared here and there in the lungs.

Gross observation at autopsy 39 rabbits had normal lung surfaces with out changes most of them being slightly uraemic non overhydrated rabbits. A few red or brown dots and spots were seen on the lung surfaces of 32 rabbits, most of which were highly uraemic. Microscopical examination showed focal haemorrhages. 13 overhydrated rabbits with in most cases profuse pleural effusion had bluish red atelectatic lungs. In several rabbits in the two last mentioned groups numerous white dots were seen on the lung surfaces.

Gross evidence of pulmonary oedema was present in 30 animals 13 of these had slight generalized changes in the form of numerous red dots or slightly diffuse spotty markings, and/or localized changes in the form of reddish brown, in most cases numerous,

spots basally or in the hilar region or spotty markings in a small part of the lung 11 rabbits had moderate macroscopical changes in the form of numerous large irregular spots, innumerable small red dots or mottled lung surfaces seen only in the lower lobes or parts thereof. In the 12 rabbits with the most extensive macroscopical changes the lungs had mottled surfaces of varying colour combinations, for instance purple—beige blue—brown red—brown—white red—pink. In 29 out of the above said 30 rabbits the macroscopical diagnosis of pulmonary oedema was further supported by at least two other criteria. All the rabbits with pulmonary oedema by at least three criteria had also gross evidence of pulmonary oedema (Diagram 18).

Frothy fluid in the air ways was found in 23 animals 1 of these had bronchitis and the rest had pulmonary oedema 7 animals with at least three criteria of pulmonary oedema had no frothy fluid in the air ways.

The lung weight was increased in 26 animals. In 4 cases it was within normal range although at least three other criteria of pulmonary oedema were present (Tables 20 and 21) In these cases the changes were localized in a small part of the lung.

The water content of the lung was increased in 34 animals. 7 animals with pulmonary oedema had normal water contents 12 without three criteria of pulmonary oedema had increased water contents (Tables 20 and 21) The average water content of the lung was much higher in overhydrated than in

5 5

III. Anesthetics 18 hours after ligature of ureters		IV. Anesthetics 72 hours after ligature of ureters	
mmHg		mmHg	
161	718	161	718
156	684	156	684
157	678	157	678
143	657	143	657
145	651	145	651
139	629	139	629
137	627	137	627
131	617	131	617
127	607	127	607
124	600	124	600
123	598	123	598
121	593	121	593
119	587	119	587
117	581	117	581
115	578	115	578
113	573	113	573
112	572	112	572
110	568	110	568
107	564	107	564
107	563	107	563
106	562	106	562
105	561	105	561
104	560	104	560
104	559	104	559
103	558	103	558
103	557	103	557
102	556	102	556
102	555	102	555
101	554	101	554
101	553	101	553
100	552	100	552
100	551	100	551
99	550	99	550
99	549	99	549
98	548	98	548
98	547	98	547
97	546	97	546
97	545	97	545
96	544	96	544
96	543	96	543
95	542	95	542
95	541	95	541
95	540	95	540
94	539	94	539
94	538	94	538
93	537	93	537
93	536	93	536
92	535	92	535
92	534	92	534
91	533	91	533
91	532	91	532
90	531	90	531
90	530	90	530
89	529	89	529
89	528	89	528
88	527	88	527
88	526	88	526
87	525	87	525
87	524	87	524
86	523	86	523
86	522	86	522
85	521	85	521
85	520	85	520
84	519	84	519
84	518	84	518
83	517	83	517
83	516	83	516
82	515	82	515
82	514	82	514
81	513	81	513
81	512	81	512
80	511	80	511
80	510	80	510
79	509	79	509
79	508	79	508
78	507	78	507
78	506	78	506
77	505	77	505
77	504	77	504
76	503	76	503
76	502	76	502
75	501	75	501
75	500	75	500
74	499	74	499
74	498	74	498
73	497	73	497
73	496	73	496
72	495	72	495
72	494	72	494
71	493	71	493
71	492	71	492
70	491	70	491
70	490	70	490
69	489	69	489
69	488	69	488
68	487	68	487
68	486	68	486
67	485	67	485
67	484	67	484
66	483	66	483
66	482	66	482
65	481	65	481
65	480	65	480
64	479	64	479
64	478	64	478
63	477	63	477
63	476	63	476
62	475	62	475
62	474	62	474
61	473	61	473
61	472	61	472
60	471	60	471
60	470	60	470
59	469	59	469
59	468	59	468
58	467	58	467
58	466	58	466
57	465	57	465
57	464	57	464
56	463	56	463
56	462	56	462
55	461	55	461
55	460	55	460
54	459	54	459
54	458	54	458
53	457	53	457
53	456	53	456
52	455	52	455
52	454	52	454
51	453	51	453
51	452	51	452
50	451	50	451
50	450	50	450
49	449	49	449
49	448	49	448
48	447	48	447
48	446	48	446
47	445	47	445
47	444	47	444
46	443	46	443
46	442	46	442
45	441	45	441
45	440	45	440
44	439	44	439
44	438	44	438
43	437	43	437
43	436	43	436
42	435	42	435
42	434	42	434
41	433	41	433
41	432	41	432
40	431	40	431
40	430	40	430
39	429	39	429
39	428	39	428
38	427	38	427
38	426	38	426
37	425	37	425
37	424	37	424
36	423	36	423
36	422	36	422
35	421	35	421
35	420	35	420
34	419	34	419
34	418	34	418
33	417	33	417
33	416	33	416
32	415	32	415
32	414	32	414
31	413	31	413
31	412	31	412
30	411	30	411
30	410	30	410
29	409	29	409
29	408	29	408
28	407	28	407
28	406	28	406
27	405	27	405
27	404	27	404
26	403	26	403
26	402	26	402
25	401	25	401
25	400	25	400
24	399	24	399
24	398	24	398
23	397	23	397
23	396	23	396
22	395	22	395
22	394	22	394
21	393	21	393
21	392	21	392
20	391	20	391
20	390	20	390
19	389	19	389
19	388	19	388
18	387	18	387
18	386	18	386
17	385	17	385
17	384	17	384
16	383	16	383
16	382	16	382
15	381	15	381
15	380	15	380
14	379	14	379
14	378	14	378
13	377	13	377
13	376	13	376
12	375	12	375
12	374	12	374
11	373	11	373
11	372	11	372
10	371	10	371
10	370	10	370
9	369	9	369
9	368	9	368
8	367	8	367
8	366	8	366
7	365	7	365
7	364	7	364
6	363	6	363
6	362	6	362
5	361	5	361
5	360	5	360
4	359	4	359
4	358	4	358
3	357	3	357
3	356	3	356
2	355	2	355
2	354	2	354
1	353	1	353
1	352	1	352
0	351	0	351
0	350	0	350
0	349	0	349
0	348	0	348
0	347	0	347
0	346	0	346
0	345	0	345
0	344	0	344
0	343	0	343
0	342	0	342
0	341	0	341
0	340	0	340
0	339	0	339
0	338	0	338
0	337	0	337
0	336	0	336
0	335	0	335
0	334	0	334
0	333	0	333
0	332	0	332
0	331	0	331
0	330	0	330
0	329	0	329
0	328	0	328
0	327	0	327
0	326	0	326
0	325	0	325
0	324	0	324
0	323	0	323
0	322	0	322
0	321	0	321
0	320	0	320
0	319	0	319
0	318	0	318
0	317	0	317
0	316	0	316
0	315	0	315
0	314	0	314
0	313	0	313
0	312	0	312
0	311	0	311
0	310	0	310
0	309	0	309
0	308	0	308
0	307	0	307
0	306	0	306
0	305	0	305
0	304	0	304
0	303	0	303
0	302	0	302
0	301	0	301
0	300	0	300
0	299	0	299
0	298	0	298
0	297	0	297
0	296	0	296
0	295	0	295
0	294	0	294
0	293	0	293
0	292	0	292
0	291	0	291
0	290	0	290
0	289	0	289
0	288	0	288
0	287	0	287
0	286	0	286
0	285	0	285
0	284	0	284
0	283	0	283
0	282	0	282
0	281	0	281
0	280	0	280
0	279	0	279
0	278	0	278
0	277	0	277
0	276	0	276
0	275	0	275
0	274	0	274
0	273	0	273
0	272	0	272
0	271	0	271
0	270	0	270
0	269	0	269
0	268	0	268
0	267	0	267
0	266	0	266
0	265	0	265
0	264	0	264
0	263	0	263
0	262	0	262
0	261	0	261
0	260	0	260
0	259	0	259
0	258	0	258
0	257	0	257
0	256	0	256
0	255	0	255
0	254	0	254
0	253	0	253
0	252	0	252
0	251	0	251
0	250	0	250
0	249	0	249
0	248	0	248
0	247	0	247
0	246	0	246
0	245	0	245
0	244	0	244
0	243	0	243
0	242	0	242
0	241	0	241
0	240	0	240
0	239	0	239

Table 20 Results after *Narkotal* anaesthesia in anuric overhydrated rabbits whose ureters had been tied 2 24 48 and 72 hours respectively before the anaesthesia K=artificial respiration L=at least three criteria of lung oedema S=spontaneous death

	Lung weight g/kg body weight	Water content in lung %	Pulmonary oedema by gross observation at 24 h	Frothy fluid in trachea	Alveolar oedema	Lung changes	X ray Cardiac enlargement	Broncholiths	Pleural fluid ml	Axillae ml	H P N mg per 100 ml	Length of ear, l after anaesthesia hours
I. Anaesthesia 2 hours after ligation of ureters	5.13	81.7	0	0	0	0	0	(+)	0	0	193	20
	5.12	81.4	0	0	0	0	0	0	0	1.4	113	25
	4.45	82.1	0	0	—	0	0	—	0	0	148	26
	4.37	83.1	0	0	0	0	0	(+)	0	0	110	24
	4.22	79.3	0	0	0	0	0	0	0	0.5	150	21
	4.16	82.0	0	0	—	0	0	—	0	2	115	23
	3.88	81.3	0	0	—	0	0	—	0	0	188	24
	3.62	81.5	0	0	—	0	0	—	0	0	150	24
	3.75	80.2	0	0	—	0	0	—	0	0	137	25
	3.59	81.2	0	0	—	0	0	—	0	0	127	25
Mean	4.55	81.4								5	139	
II. Anaesthesia 24 hours after ligation of ureters	4.92	80.5	0	0	0	0	0	0	1	110	220	25
	4.69	81.3	0	0	0	0	0	0	0	3	151	26
	4.27	82.6	0	0	+	+	0	+	0	1	210	25
	4.23	82.1	0	0	—	0	0	—	0	0	203	26
	4.70	80.7	0	0	—	0	0	—	0	0	185	26
	4.17	80.8	0	0	—	0	0	—	0	0	181	25
	4.12	82.1	0	0	0	0	0	(+)	0	10	181	23
	3.93	81.5	0	0	—	0	0	—	1	10	185	26
	3.91	80.9	0	0	—	0	0	—	1	0	186	24
	3.31	81.0	0	0	—	0	0	—	0	57	108	26
Mean	4.15	81.7							5	3	153	27
									0.5	19		

**三三三**

III. Amplitude is lower after ligation of artery

一、

U. S. DEPARTMENT OF AGRICULTURE  
BUREAU OF PLANT INDUSTRY  
WASHINGTON, D. C.

U.S. 9-1-1

[illegible]

Table 21 Results after *Narkotol* anaesthesia and infusion of Ringer's solution amounting to 15% of the body-weight for 1 1/2 hours in anuric rabbits whose ureters had been tied 2, 48 and 72 hours respectively before the anaesthesia  
 A = artificial respiration, L = at least three criteria of lung oedema, S = spontaneous death

	Lung weight g/g body weight	Water content in lung %	Pulmonary oedema by gross observ.	Frothy fluid in the alveoli	Alveolar oedema	X ray	Cardiac enlargement	Bronchitis	Pleural fluid ml	Aseptic ml	N P V. mg per 100 ml	Length of survival after anaesthesia hours
K	0.07	82.5	+	0	+	+	+	+	5	5	107	25
	0.18	82.2	0	0	0	+	+	0	3	2	158	25
	0.36	83.5	0	0	0	0	0	+	12	25	180	20
	0.31	83.8	+	+	0	+	+	0	7	20	87	26
K	0.23	82.8	0	+	+	+	+	+	12	7	117	25
	0.14	82.9	+	0	0	+	+	0	27	6	97	26
	0.03	82.5	0	0	0	+	+	0	0	0	98	26
	0.01	82.1	0	0	0	+	+	—	5	85	104	26
K	0.01	82.9	0	0	0	0	+	0	7	10	110	25
K	0.07	83.2	0	0	0	+	+	0	17	55	83	20
A	0.05	83.5	0	0	0	+	+	+	7	3	174	25
	0.78	83.0	0	0	0	+	+	+	32	30	82	23
	0.72	82.4	0	0	+	+	+	(+)	23	22	142	25
	0.07	81.5	0	0	0	+	+	0	8	12	107	25
A	0.03	83.1	0	0	0	0	+	0	34	33	81	26
	0.44	82.9	0	0	0	+	+	0	31	63	121	23
	0.42	83.3	0	0	0	+	+	—	7	4	98	20
	0.23	82.9	0	0	0	+	+	0	20	25	152	25
A	0.22	82.5	0	0	0	+	+	(+)	41	52	121	26
	0.06	83.3	0	0	+	+	+	—	15	10	126	25
	0.63	82.7	0	0	0	0	0	0	44	31	92	25
	0.52	81.8	0	0	0	+	+	0	26	163	78	20
Mean	0.77	83.2	0	0	—	0	0	—	18	32	112	

I Anaesthesia = hours after ligation of ureters

### III. *Ascaris* 48 hours after ligature of ureters

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

weight  
after ligature of ureters  
II. *Anastrepha* 24 hours

Table 21 Results after *Narkotal* anaesthesia and infusion of Ringer's solution amounting to 15% of the body-weight for 1 1/2 hours in anuric rabbits whose ureters had been tied 2, 24, 48 and 72 hours respectively before the anaesthesia  
 A = artificial respiration, L = at least three criteria of lung oedema, S = spontaneous death

	Lung weight g/kg body weight	Water content of lung %	Pulmonary oedema by gross weight (than air way)	Prothym fluid in the	Alveolar oedema	Lung changes	X ray	Cardia enlargement	Bronchio-liti	Pleural fluid ml	Asthesia m	Y P V mg per 100 m	Length of survival after anaesthesia hours
A	L 13.06	88.0	++	++	+	++		+	+	4	31	241	8 S
	L 11.97	84.6	++	++	0	++	+	+	0	16	175	315	4 S
	L 11.79	87.3	++	++	+	++	+	+	+	4	82	345	0 S
	L 10.70	89.0	++	++	+	++	+	+	0	7	48	178	3 S
L	L 9.00	89.1	++	++	+	++	+	+	0	10	40	230	2 S
	L 7.06	84.3	++	++	+	++	+	+	+	17	236	265	23 S
	L 7.47	82.6	++	++	+	++	+	+	0	12	185	281	25 S
	L 7.19	81.8	++	++	+	++	+	+	+	7	176	359	25 S
A	L 6.48	83.8	++	++	+	++	+	+	0	31	162	277	25 S
	L 5.37	80.8	++	++	+	++	0	0	+	2	376	267	23 S
	L 4.81	82.1	+	+	0	0	0	+	0	8	78	205	23 S
	L 4.78	82.3	0	0	0	0	0	0	+	3	322	310	23 S
K	L 4.02	80.7	0	0	0	0	0	0	0	2	342	301	22 S
	L 3.57	80.6	0	0	0	0	0	0	0	1	376	314	26 S
	L 3.20	81.6	+	+	0	0	0	0	+	10	202	200	25 S
	L 4.30	81.9	0	0	0	0	0	0	0	0	370	303	25 S
K	L 4.33	82.6	0	0	0	+	+	0	+	51	155	302	26 S
	L 4.08	81.1	0	0	0	0	0	0	+	14	251	280	27 S
	L 4.01	80.0	0	0	0	0	0	+	0	12	16	301	28 S
	L 4.02	81.0	0	0	0	0	0	0	0	7	113	300	23 S
Mean	L 3.77	81.0	0	0	0	0	0	0	+	1	22	333	25 S
	L 3.09	80.9	0	0	0	0	0	0	0	16	263	236	26 S
	L 3.0	83.0	0	0	0	0	0	0	0	10	381	237	25 S
	L 3.13	83.0	0	0	0	0	0	0	0	11	311	287	25 S

alight, it occurred in patches, over the hilar or basal regions. In these cases the macroscopical and microscopical pictures established the diagnosis. In 3 there were no radiographic pulmonary changes, in 4 the lung weights were normal and in 5 the water contents were normal. Atelectasis, hyperaemia haemorrhages into the lung parenchyma and pneumonia were present though in a low frequency and did not cause any very great diagnostic difficulties.

#### *Pulmonary oedema and uraemia*

It will be seen from Tables 20 and 21 and from Diagrams 8 and 9 that the frequency of pulmonary oedema as defined here was as follows

#### *A Non-overhydrated animals*

Narkotal narcosis 2 hours after ureteral ligation. The series comprised 10 animals which were bled within 24 hours of the narcosis. Pulmonary oedema was not noted in any of the cases.

Narkotal narcosis 24 hours after ureteral ligation. 10 rabbits were bled within 24 hours of the narcosis. Pulmonary oedema was not noted in any of the cases.

Narkotal narcosis 48 hours after ureteral ligation. The series comprised 10 rabbits which were bled within 24 hours of the narcosis. Pulmonary oedema was noted in 2 cases.

Originally the series included 5 other 2 animals, but they died spontaneously before the bloodletting, and as their bodies

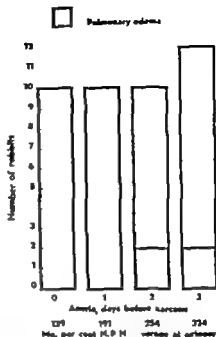


Diagram 8 Occurrence of pulmonary oedema after "Narkotal" anaesthesia in non-overhydrated rabbits which had been anuric for 0, 1, 2, and 3 days, respectively before the induction of anaesthesia.

were already cold and rigid when discovered, the presence of pulmonary oedema could not be ascertained.

Narkotal narcosis 72 hours after ureteral ligation. The series comprised 12 animals. 9 were bled within 24 hours and 1 which was moribund, within 12 hours of the narcosis. 11 died spontaneously before the bloodletting. Pulmonary oedema was noted in 2 cases.

The series included originally a further 2 animals which died spontaneously and were already cold and rigid. 1 autopsy-pulmonary oedema was probably not present.



Table 21 Results after *Yarkotal* anaesthesia and infusion of Ringer's solution amounting to 15% of the body weight for 1 1/2 hours in anæsthetic rabbits whose ureters had been tied 2 24 48 and 72 hours respectively before the anaesthesia  
A=artificial respiration L=at least three criteria of lung oedema. S=spontaneous death

	Lung weight g/kg body weight	Urinary content of lung %	Pulmonary oedema by gross body weight	Brothy fluid in 1 ways	Al collar oedema	Lung haemorrhage	X ray Carilla engorgement	Bronchio- llitis	Pleural fl uid ml	Anæsthesia ml	N P N mg per 100 ml	Length of survival after anaesthesia h. m.
K	L 13.06	83.6	++	+	+	++	+	+	4	31	241	8 S
	L 11.07	81.6	++	+	0	++	+	0	15	175	315	4 S
	L 11.70	87.2	++	++	+	++	+	+	4	62	345	0 S
	L 10.79	80.0	++	++	+	++	+	0	7	118	178	3 S
L	L 9.69	83.1	++	++	++	++	+	0	10	40	220	2 S
	L 7.96	81.2	++	+	++	++	0	+	17	200	205	25 S
	L 7.47	82.5	+	+	++	++	+	0	12	185	281	25 S
	L 7.19	81.8	+	+	++	++	0	+	7	175	250	25 S
A	L 0.18	82.8	++	0	++	++	++	0	31	162	277	25 S
	L 5.27	80.8	++	+	+	++	+	+	2	370	287	25 S
	L 1.81	82.1	+	0	0	0	+	0	8	78	205	23 S
	L 1.79	82.5	0	0	0	0	0	+	3	322	310	25 S
A	L 4.02	80.7	0	0	0	0	0	0	2	342	301	22 S
	L 4.57	80.0	0	0	0	0	0	0	1	375	341	25 S
	L 1.50	81.6	+	0	0	0	0	+	10	202	200	26 S
	L 1.37	80.8	0	0	0	0	0	0	0	370	303	25 S
K	L 4.35	81.9	0	0	0	+	0	+	54	155	302	25 S
	L 4.33	82.6	0	0	—	0	0	—	14	251	290	27 S
	L 1.09	81.1	0	0	0	0	+	0	12	178	301	28 S
	L 4.04	80.0	0	0	0	0	0	0	7	412	300	23 S
Mean	L 4.02	81.9	0	0	0	0	0	+	1	27	332	25 S
	L 3.82	81.0	0	0	0	0	0	0	18	263	238	20 S
	L 3.7	80.9	0	0	0	0	0	0	10	281	227	23 S
	L 6.12	83.0	0	0	0	0	0	0	11	311	267	

mic (at least 48 hours of anuria) overhydrated rabbits, in 18 % of the severely uraemic non-overhydrated rabbits, in 13 % of the slightly (anuria for less than 48 hours) uraemic overhydrated rabbits, and in none of the slightly uraemic non-overhydrated rabbits. The non-overhydrated rabbits and those with uraemia of low degree had only slight pulmonary oedema.

The frequency of pulmonary oedema following Narkotal® narcosis and overhydration was higher in severely (at least 48 hours of anuria) than in slightly (anuria for less than 24 hours) uraemic rabbits. The difference is significant.

7 % of the animals died during the night, at times when they were not under observation, and as autopsy was not performed until their bodies had become cold and rigid, the presence of pulmonary oedema could not be ascertained. This fact does not seem to have influenced the results of the investigation, since it was exactly the highly uraemic animals that died, and since the difference in numbers between overhydrated and non-overhydrated animals was very small.

#### *Pulmonary oedema and overhydration*

Pulmonary oedema following Narkotal® narcosis occurred more frequently among overhydrated than among non-overhydrated uraemic rabbits whose ureters had been ligated. This observation refers to all degrees of uraemia but the difference was greatest for severely uraemic rabbits ( $p < 0.05$ ).

#### *Pulmonary oedema and depth and duration of narcosis*

The frequency of pulmonary oedema as defined here has been calculated for 120 overhydrated or non-overhydrated rabbits which were anaesthetized with "Narkotal". During the anaesthesia 29 of these rabbits had respiratory standstill which lasted long enough to necessitate artificial respiration; pulmonary oedema developed in 31 % of this group. During the anaesthesia 69 rabbits had a minimum respiratory rate of 4—18 per minute; pulmonary oedema developed in 17 % of these cases. In 22 rabbits the minimum respiratory rate was 20 per minute; pulmonary oedema developed in 36 %.

59 rabbits were anaesthetized with Narkotal for 30 minutes or less; pulmonary oedema developed in 24 %. Among 61 rabbits anaesthetized for longer than 30 minutes, pulmonary oedema arose in 25 %.

Accordingly these results lend no support to the assumption that the frequency of pulmonary oedema under the experimental conditions used here would be related to the depth and the duration of anaesthesia.

#### *Microscopical findings notably bronchiolitis*

Bleedings occurred in 5 % of the examined lungs, predominantly as focal small haemorrhages but also as diffuse occurrence of red cells in alveoli accompanied by an increase of alveolar phagocytes. Large haemorrhages occurred in rabbits with pulmonary oedema, but only in a few

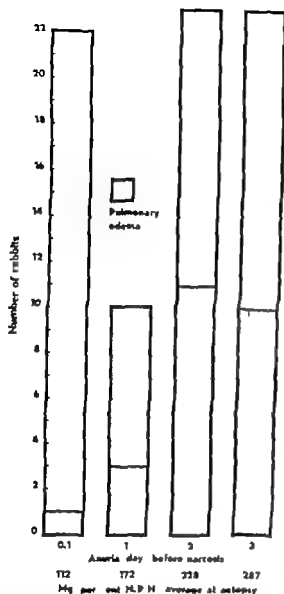


Diagram 9 Occurrence of pulmonary edema after 'Narkotal' anaesthesia and intravenous infusion of Ringer's solution, 15% of the body weight for 1 1/2 hours, in rabbits which had been anuric for 0.1, 1, 2, and 3 days, respectively before induction of anaesthesia.

### B Overhydrated animals

Narkotal narcosis and overhydration 2 hours after ureteral ligation The series comprised 22 rabbits which were bled within 24 hours of the narcosis.

Slight pulmonary oedema was noted in 1 case

Originally the series included 1 more rabbit which died spontaneously after the infusion autopsy was performed too late for ascertainment of the presence of pulmonary oedema

Narkotal narcosis and overhydration 24 hours after ureteral ligation The series comprised 10 animals which were bled within 24 hours of the narcosis Slight pulmonary oedema was noted in 3 cases.

Narkotal narcosis and overhydration 48 hours after ureteral ligation Among the 23 animals in the series, 16 were bled within 24 hours of the narcosis and 7 died spontaneously before the bloodletting Pulmonary oedema was noted in 11 cases.

The series included 1 more rabbit which died spontaneously as the body was cold and rigid at autopsy the presence of pulmonary oedema could not be ascertained

Narkotal narcosis and overhydration 72 hours after ureteral ligation The series comprised 23 animals 18 were bled within 24 hours of the narcosis, and 5 died spontaneously before the bloodletting Pulmonary oedema was noted in 10 cases.

The series included 3 more rabbits which died spontaneously as the bodies were cold and rigid at autopsy the presence of pulmonary oedema could not be ascertained

Pulmonary oedema developed within 24 hours in 40% of the severely uraemic

## Pulmonary oedema following injection of adrenaline

Pulmonary oedema following injection of adrenaline in experimental animals was first described by Bonchard & Claude in 1902 and has later been reported by many other authors (e.g. 78, 133, 183). Several surveys of the literature on the subject have been published (69, 114, 168, 211).

Adrenaline produces lung oedema in rabbits, guinea-pigs, and mice. Dogs and cats die from suffocation or heart failure without pulmonary oedema (69). Pulmonary oedema follows injection administered intravenously (e.g. 167), intramuscularly (68), subcutaneously (33), intraperitoneally (209) or intratracheally (25). The individual variations are great. After a certain dose some animals survive without developing lung oedema, in some lung oedema follows, and others die without developing lung oedema (e.g. 167, 183, 211, 237). Drenchhahn (69) noted pulmonary oedema, diagnosed by increased lung weight, in half the rabbits 15–30 minutes after intramuscular injection of 3 mg of adrenaline per kg body weight; the pressure in the pulmonary vein rose rapidly within 2 minutes. Brumm (42) without giving any other data, stated that adrenaline can cause pulmonary oedema even in nephrectomized rabbits.

The generation of pulmonary oedema after injection of adrenaline has been presumed to be increased left ventricular work due to arterial vasoconstriction, which leads to relative cardiac insufficiency

and shift of the blood volume from the systemic to the pulmonary circulation (46, 69, 168, 202, and others). In the rabbit, the pulmonary venous pressure is raised proportionally to the lung weight (68).

Kramer (1933) considered that the increased blood-volume in the lungs was attributable to a vasomotor redistribution of blood rather than to left ventricular failure.

The central nervous system is thought to play a part in the genesis of adrenaline lung oedema. Accordingly cervical vagotomy (167) or sympathectomy unilaterally or brain-stem lesions (99) protect against the development of pulmonary oedema. Diverse pharmacological preparations, such as chloral hydrate, barbiturates, heparin, morphine, palfium, phenergan, and others, have been found to exert some protective action against lung oedema (24, 114, 167, 200, 210). Stone & Loew (1949) however, believed that the susceptibility to lung oedema after adrenaline increased when barbiturates had been given immediately before the injection. Analeptics (167) and vagotomy (25) increased the susceptibility to pulmonary oedema.

Bronchial constriction following large doses of adrenaline has been considered as a cause of death in pulmonary oedema (25). Lung oedema develops less frequently in tracheotomized than in non-tracheotomized experimental animals (243). The development of lung oedema after injection of adrenaline is counteracted by artificial respiration (80) and can be prevented by lowering the negative pressure in the chest (137).

cases without pulmonary oedema. The frequency of bleedings increased in significantly with increasing uraemia.

Atelectasis was a common finding in blue-pink small lungs or lung lobes. Extensive changes were noted in 11 and less extensive changes in 10 cases. The amount of pleural effusion in these 21 rabbits averaged 26 (0—54) ml. Atelectasis was commonest in rabbits with slight uraemia. Local atelectasis occurred in rabbits with bronchitis. In the rabbit which had 637 ml of ascitic fluid there were no atelectasis or other microscopical abnormalities.

An increase in the number of polymorphonuclear leucocytes was noticed in 10 cases. Areas of purulent bronchitis were seen in 5 and small pneumonic lesions in 2 cases. These animals had not been given antibiotics.

One rabbit which died shortly after the infusion had periarterial oedema.

Bronchiolitis (Figs 18 and 19) was histologically the most striking change in these uraemic rabbits after Narkotal anaesthesia and was not noted in any other category of the animals in this investigation. The lesions were confined to the small bronchioles.

The bronchiolitis was characterized by destruction of the epithelial cells and splitting of the nuclei into minute fragments. In some cases it involved only a few bronchioles or perhaps only a small part of the wall in a bronchiole. This finding is designated as "+" in the table. Fibrinous membranes were in a few cases seen in the bronchioles, extending towards the alveoli. Hyaline membranes were faintly discernible in 1 case.

Lungs from 108 uraemic non-overhydrated or overhydrated rabbits which had received 1.7 ml or 1.2 ml of Narkotal per kg body weight intravenously were examined microscopically. Bronchiolitis was present in 51%. 45 animals had distinct and 11 had less marked changes. Among the 45 rabbits with distinct bronchiolitis 44% had pulmonary oedema. Out of 63 rabbits without or with insignificant bronchiolitis 11 (17%) had pulmonary oedema. 7 out of these 11 died spontaneously however within a few hours of the narcosis. Bronchiolitis was not present in rabbits which died within 8 hours of the narcosis. 91 rabbits with pulmonary oedema survived at least 20 hours after the narcosis. 86% had distinct bronchiolitis. Out of 77 rabbits without at least three criteria of pulmonary oedema 33% had distinct bronchiolitis. Bronchiolitis was present in 33% of 22 examined animals which had had anuria for 2 hours before the narcosis, in 40% of 20 animals with anuria for 24 hours, in 61% of 33 animals with anuria for 48 hours, and in 58% of 33 animals with anuria for 72 hours. There was no correlation between the duration of anaesthesia and the occurrence of bronchiolitis ( $r=0.03 \pm 0.10$ ) nor between maximum depth of narcosis and the occurrence of bronchiolitis ( $r=0.20 \pm 0.09$ ).

Whether the bronchiolitis was a parallel phenomenon or a prerequisite for pulmonary oedema is, thus, not clear.

*Survey. See page 149*

rabbits a) Among 10 animals which had been anuric for 3—4 hours before the injection of adrenaline, none died within 20 minutes and 2 within 65 minutes 8 were bled. b) Among 10 animals which had been anuric for 48 hours before injection of adrenaline 4 died within 20 minutes and none within 3 hours 5 were bled. c) Out of 10 animals which had been anuric for 72 hours before adrenaline injection 3 died within 20 minutes and 5 within 3 hours 2 were bled.

In the sold groups the mean survival time was shortest (54 minutes) for the non-overhydrated animals with anuria for 72 hours and longest (206 minutes) for overhydrated animals with anuria for 3—4 hours (Tables 22 and 23)

#### *Symptoms in vivo*

For each group the results could have been grouped and reported under different headings, for instance pulmonary oedema—no pulmonary oedema slight uraemia—severe uraemia, overhydration,—non-overhydration. Since such an account would take up too much space, the groups will be brought together when they are similar or of little interest individually. The frequency of pulmonary oedema in the different groups will be reported after the symptoms in vivo and autopsy findings.

**Appearance and behaviour.** Within a few minutes of the injection of adrenaline the animals became restless and moved about in the room all the time, often sniffing keenly and seemingly frightened. They had periods of tachypnoea and dyspnoea.

The subsequent course varied individually. Out of 70 rabbits, 13 died within 25 minutes and 4 within 1 hour of the injection, with frothy fluid gushing forth from the nose and the mouth the flow and the froth were less profuse in the overhydrated than in the non-overhydrated animals. In severely uraemic rabbits the course from injection up to death was quieter and the amount of frothy fluid less than in the slightly uraemic rabbits and the controls.

Convulsions immediately before death occurred in about half the cases and both in seemingly moribund and in seemingly alert animals. The occurrence of convulsions was not correlated to the occurrence of pulmonary oedema.

Weakness developed in about one-fourth of the rabbits within a few minutes of the injection of adrenaline and later in most of those which survived the first hour of restlessness death occurred inconspicuously in most of these animals. The weakness was particularly marked in highly uraemic rabbits. In cases of great weakness it was very difficult to judge from the animal's condition whether or not it had pulmonary oedema. Even rabbits which survived until they were bled would sit still or lie flat on the floor during the last few hours before the bloodletting. Serum potassium was determined in 9 animals at the bloodletting the levels did not differ significantly from those in anuric animals which had not had injections of adrenaline.

**Respiratory rate.** In 7 normal rabbits

Adrenaline did not induce pulmonary oedema in experiments with isolated lungs of rabbits (40). Other pharmacological preparations, for instance diisotrophenol, mercury compounds, and ammonia induced lung oedema in such experiments, which was interpreted as a result of permeability damage.

Blood accumulating in the lungs has been noted in the initial stage of adrenaline-induced pulmonary oedema (211).

Wiggers produced lung oedema in dogs by infusion of adrenaline and saline (Chapter 8).

### *Own investigations*

This series was used for the purpose of studying the effect of injection of adrenaline in control animals and non-overhydrated and overhydrated rabbits with ligated ureters and uraemia of varying duration.

The series comprised 10 controls, and 80 rabbits whose ureters had been tied bilaterally. The latter were divided into 3 groups of 20 animals: adrenaline was injected 1—4, 48, and 72 hours respectively after the operation. In half the rabbits of each group Ringer's solution was infused intravenously in amounts equalling 15% of the body weights for 1 1/2 hours. Adrenaline was injected 1 hour after the termination of infusion. In the overhydrated rabbits the dose of adrenaline was proportioned to body weight after the infusion. It was thus estimated that an approximately equal adrenaline concentration would be obtained in the body fluids of overhydrated and of non-overhydrated rabbits.

Accordingly overhydrated animals received a dose which was, on an average 15% larger than that given to the non-overhydrated ones.

Adrenaline was injected intramuscularly by Drenckhahn's (42) method. The dose was reduced, however to 1 mg per kg (1 ml 1% adrenaline solution per kg) as preliminary experiments showed that in uraemic rabbits this dose produced pulmonary oedema in a desired frequency without too high mortality. Before the injection checking was done by aspiration to make sure that the point of the needle was not in a blood vessel.

The rabbits were allowed to move freely in the room under continuous observation. Electrocardiograms were taken and the respiratory rate measured frequently after the injection. In the rabbits that died spontaneously chest radiographs were taken immediately after death. Rabbits that lived for 5 hours after the injection were bled as soon as chest radiographs and electrocardiograms had been taken and the respiratory rate had been measured. The time of 5 hours was chosen because according to preliminary experiments, the effect of adrenaline usually begins to subside after that time.

### *Survival time*

Out of 10 controls, 4 died spontaneously within 20 minutes and 4 within 5 hours. 2 were bled after 5 hours.

The series of non-overhydrated anuric rabbits: a) Out of 10 animals which had been anuric for 1—4 hours before the injection of adrenaline 3 died within 20 minutes and 1 within 70 minutes. 6 were bled after 5 hours. b) Among 10 animals which had been anuric for 48 hours before the injection of adrenaline 3 died within 20 minutes and 4 within 3 hours. 3 were bled. c) Among 10 animals which had been anuric for 72 hours before the injection of adrenaline 2 died within 20 minutes and 8 within 130 minutes.

The series of overhydrated anuric

rabbits a) Among 10 animals which had been anuric for 3—4 hours before the injection of adrenaline none died within 20 minutes and 2 within 65 minutes 8 were bled. b) Among 10 animals which had been anuric for 48 hours before injection of adrenaline, 4 died within 20 minutes and none within 5 hours 6 were bled c) Out of 10 animals which had been anuric for 72 hours before adrenaline injection, 3 died within 20 minutes and 5 within 3 hours 2 were bled.

In the said groups the mean survival time was shortest (54 minutes) for the non-overhydrated animals with anuria for 72 hours and longest (266 minutes) for overhydrated animals with anuria for 3—4 hours (Tables 22 and 23)

#### *Symptoms in vivo*

For each group the results could have been grouped and reported under different headings for instance pulmonary oedema—no pulmonary oedema, slight uraemia—severe uraemia, overhydration,—non-overhydration. Since such an account would take up too much space, the groups will be brought together when they are similar or of little interest individually. The frequency of pulmonary oedema in the different groups will be reported after the symptoms in vivo and autopsy findings.

**Appearance and behaviour.** Within a few minutes of the injection of adrenaline the animals became restless and moved about in the room all the time often sniffing keenly and seemingly frightened. They had periods of tachypnoea and dyspnoea.

The subsequent course varied individually. Out of 70 rabbits, 13 died within 25 minutes and 4 within 1 hour of the injection, with frothy fluid gushing forth from the nose and the mouth the flow and the froth were less profuse in the overhydrated than in the non-overhydrated animals. In severely uraemic rabbits the course from injection up to death was quieter and the amount of frothy fluid less than in the slightly uraemic rabbits and the controls.

Convulsions immediately before death occurred in about half the cases and both in seemingly moribund and in seemingly alert animals. The occurrence of convulsions was not correlated to the occurrence of pulmonary oedema.

Weakness developed in about one-fourth of the rabbits within a few minutes of the injection of adrenaline and later in most of those which survived the first hour of restlessness death occurred inconspicuously in most of these animals. The weakness was particularly marked in highly uraemic rabbits. In cases of great weakness it was very difficult to judge from the animal's condition whether or not it had pulmonary oedema. Even rabbits which survived until they were bled would sit still or lie flat on the floor during the last few hours before the bloodletting. *Serum potassium* was determined in 9 animals at the bloodletting: the levels did not differ significantly from those in anuric animals which had not had injections of adrenaline.

**Respiratory rate.** In 7 normal rab-



Adrenaline did not induce pulmonary oedema in experiments with isolated lungs of rabbits (40). Other pharmacological preparations, for instance dinitrophenol, mercury compounds, and ammonia induced lung oedema in such experiments which was interpreted as a result of per meability damage.

Blood accumulating in the lungs has been noted in the initial stage of adrenaline-induced pulmonary oedema (211).

Wiggers produced lung oedema in dogs by infusion of adrenaline and saline (Chapter 6).

### *Own investigations*

This series was used for the purpose of studying the effect of injection of adrenaline in control animals and non-overhydrated and overhydrated rabbits with ligated ureters and uraemia of varying duration.

The series comprised 10 controls, and 60 rabbits whose ureters had been tied bilaterally. The latter were divided into 3 groups of 20 animals: adrenaline was injected 1—4, 48, and 72 hours, respectively after the operation. In half the rabbits of each group Ringer's solution was infused intravenously in amounts equalling 15% of the body weights for 1 1/2 hours. Adrenaline was injected 1 hour after the termination of infusion. In the overhydrated rabbits the dose of adrenaline was proportioned to body weight after the infusion. It was thus estimated that an approximately equal adrenaline concentration would be obtained in the body fluids of overhydrated and of non-overhydrated rabbits.

Accordingly overhydrated animals received a dose which was on an average 15% larger than that given to the non-overhydrated ones.

Adrenaline was injected intramuscularly by Drenckhahn's (42) method. The dose was reduced, however to 1 mg per kg (1 ml 1% adrenaline solution per kg) as preliminary experiments showed that in uraemic rabbits this dose produced pulmonary oedema in a desired frequency without too high mortality. Before the injection checking was done by aspiration to make sure that the point of the needle was not in a blood vessel.

The rabbits were allowed to move freely in the room under continuous observation. Electrocardiograms were taken and the respiratory rate measured frequently after the injection. In the rabbits that died spontaneously chest radiographs were taken immediately after death. Rabbits that lived for 5 hours after the injection were bled as soon as chest radiographs and electrocardiograms had been taken and the respiratory rate had been measured. The time of 5 hours was chosen because according to preliminary experiments, the effect of adrenaline usually begins to subside after that time.

### *Survival time*

Out of 10 controls, 4 died spontaneously within 20 minutes and 4 within 5 hours. 2 were bled after 5 hours.

The series of non-overhydrated anuric rabbits: a) Out of 10 animals which had been anuric for 1—4 hours before the injection of adrenaline 3 died within 20 minutes and 1 within 70 minutes. 6 were bled after 5 hours. b) Among 10 animals which had been anuric for 48 hours before the injection of adrenaline 2 died within 20 minutes and 4 within 3 hours. 4 were bled. c) Among 10 animals which had been anuric for 72 hours before the injection of adrenaline 2 died within 20 minutes and 8 within 130 minutes.

The series of overhydrated anuric

rabbits a) Among 10 animals which had been anuric for 3—4 hours before the injection of adrenaline, none died within 20 minutes and 2 within 65 minutes 8 were bled. b) Among 10 animals which had been anuric for 48 hours before injection of adrenaline 4 died within 20 minutes and none within 5 hours 6 were bled. c) Out of 10 animals which had been anuric for 72 hours before adrenaline injection, 3 died within 20 minutes and 5 within 3 hours 2 were bled.

In the said groups the mean survival time was shortest (54 minutes) for the non-overhydrated animals with anuria for 72 hours and longest (266 minutes) for overhydrated animals with anuria for 3—4 hours (Tables 22 and 23)

#### *Symptoms in vivo*

For each group the results could have been grouped and reported under different headings, for instance pulmonary oedema—no pulmonary oedema slight uraemia—severe uraemia, overhydration,—non-overhydration. Since such an account would take up too much space the groups will be brought together when they are similar or of little interest individually. The frequency of pulmonary oedema in the different groups will be reported after the symptoms in vivo and autopsy findings.

**Appearance and behaviour** Within a few minutes of the injection of adrenaline the animals became restless and moved about in the room all the time often sniffing keenly and seemingly frightened. They had periods of tachypnoea and dyspnoea

The subsequent course varied individually. Out of 70 rabbits, 13 died within 25 minutes and 4 within 1 hour of the injection, with frothy fluid gushing forth from the nose and the mouth the flow and the froth were less profuse in the overhydrated than in the non-overhydrated animals. In severely uraemic rabbits the course from injection up to death was quieter and the amount of frothy fluid less than in the slightly uraemic rabbits and the controls.

**Convulsions** Immediately before death occurred in about half the cases and both in seemingly moribund and in seemingly alert animals. The occurrence of convulsions was not correlated to the occurrence of pulmonary oedema.

**Weakness** developed in about one-fourth of the rabbits within a few minutes of the injection of adrenaline and later in most of those which survived the first hour of restlessness death occurred inconspicuously in most of these animals. The weakness was particularly marked in highly uraemic rabbits. In cases of great weakness it was very difficult to judge from the animal's condition whether or not it had pulmonary oedema. Even rabbits which survived until they were bled would sit still or lie flat on the floor during the last few hours before the bloodletting. Serum-potassium was determined in 9 animals at the bloodletting the levels did not differ significantly from those in anuric animals which had not had injections of adrenaline.

**Respiratory rate** In 7 normal rab-

bility the respiratory rate was measured at short intervals from the adrenaline injection up to death which occurred within 35—310 minutes after the injection. Four of these animals had at least three criteria of pulmonary oedema at autopsy. The respiratory rate varied greatly from minute to minute but seldom exceeded 200 per minute. Attacks of dyspnoea occurred. Despite these respiratory studies, however it was not possible to establish with any degree of certainty which animals had or were to develop pulmonary oedema.

In 28 anuric rabbits, which later on did not exhibit pulmonary oedema the respiratory rate about 30 minutes after adrenaline injection averaged 109 (36—320) per minute. In 27 rabbits which later on exhibited pulmonary oedema the respiratory rate averaged 187 (72—380) per minute. The severely uraemic animals with pulmonary oedema had a respiratory rate as high as 242 (120—380) per minute.

In 23 severely ill uraemic rabbits the respiratory rate was recorded within 10 minutes before spontaneous death. In 15 rabbits with pulmonary oedema it averaged 228 (92—380) and in 8 rabbits without pulmonary oedema 97 (26—200) per minute.

The respiratory rate was thus of some aid in establishing or excluding the diagnosis of pulmonary oedema following adrenaline injection in all the groups and hence in severely uraemic animals as well. However the individual variations in the frequency of tachypnoea and dyspnoea reduced the diagnostic value of the symptoms in the individual case.

E.C.G. and heart rate. In 10 normal and 4 anuric rabbits electrocardiograms were taken at short intervals up to death. Bradycardia arose within some minutes of the adrenaline injection. After a further few minutes deformation of the QRS-complex and/or arrhythmia appeared. Out of 8 rabbits that died in this stage, 4 had pulmonary oedema. In the 6 animals that lived longer arrhythmia and QRS deformation also appeared but the symptoms subsided or disappeared later on and the bradycardia changed into tachycardia. Tachycardia and flat or negative T waves persisted in many cases up to a few minutes before spontaneous death when the frequency of severe electrocardiographic changes increased again.

Thus in this group despite frequent recordings, the development of pulmonary oedema could not be predicted with the aid of E.C.G. The animals could have severe E.C.G. disturbances for several hours before spontaneous death and yet exhibit no evidence of pulmonary oedema at autopsy. All those that died spontaneously had severe E.C.G. abnormalities immediately before death.

Before the adrenaline injection 29 out of 27 subsequently bled rabbits had normal electrocardiograms, whereas in 5 the T waves were absent. Five hours after the adrenaline injection the E.C.G. showed no abnormalities in 6, absent T waves in 5, negative T waves in 17 and deformation of the QRS-complex in 1.

The heart rate of the 27 bled rabbits averaged before the adrenaline injection

tion 237 (120—330) and 5 hours after the injection 235 (136—275) beats per minute. 7 rabbits with pulmonary oedema had immediately before the bloodletting an average heart rate of 235 (170—273) beats per minute.

All the rabbits with pulmonary oedema had more or less severe E.C.G. changes, but, again, many animals without pulmonary oedema had similar changes. The heart rate showed the same variations in animals with and without pulmonary oedema. Rabbits in all stages of uraemia had severe E.C.G. disturbances after the adrenaline injection. In the highly uraemic ones the changes disappeared less readily or persisted. The E.C.G. showed the same disturbances in the overhydrated as in the non-overhydrated animals.

Thus, the E.C.G. and the heart rate were of little diagnostic value in the adrenaline series.

**Heart size** The series consisted of 70 animals. Before the adrenaline injection the heart-size could be estimated radiographically in 62. In 8 the heart could not be assessed for technical reasons or because pictures were missing. In 55 of the 62 animals the heart size was normal, whereas 8 (all overhydrated) had slight cardiac enlargement.

After the adrenaline injection immediately before bloodletting spontaneous death, and autopsy respectively (Tables 22 and 23) the results for the 70 rabbits were as follows. The heart was markedly enlarged in 3, moderately enlarged in 13 slightly enlarged in 19 and of normal size in 24

cases the heart size could not be assessed in 10 rabbits in which pulmonary oedema made definition of the heart impossible the radiograph was missing in 1 case. If the 70 animals (10 normal and 60 uraemic) are grouped by overhydration or non-overhydration and severe, slight, or no uraemia, the following figures will be obtained. In 25 of 30 overhydrated uraemic rabbits the heart could be defined 72 % had cardiac enlargement. In 34 out of 40 non-overhydrated rabbits (30 uraemic and 10 normal) the heart could be defined 50 % had cardiac enlargement. In 24 of 30 normal or slightly uraemic (anuria for less than 24 hours) rabbits the heart could be defined 71 % had cardiac enlargement. In 35 out of 40 highly uraemic (anuria for 48 hours) rabbits the heart could be defined 51 % had cardiac enlargement. Cardiac enlargement was commonest in overhydrated rabbits with slight uraemia.

14 out of 70 rabbits were not examined until immediately after death. In 8 the heart was of normal size, in 3 slightly enlarged, and in 3 moderately enlarged. If these are taken into account, 63 % of 24 animals with pulmonary oedema had no or slight cardiac enlargement and 56 % of 16 animals with marked to moderate cardiac enlargement had pulmonary oedema.

The ratio pulmonary oedema/cardiac enlargement could be studied in 25 bled animals (Tables 22 and 23). 9 had normal heart size (2 had pulmonary oedema) 5 had slightly enlarged heart (2 had pulmonary oedema) 11 had moderately enlarged heart (2 had pul

Table 22 Results after intramuscular injection of adrenaline 1 mg per kg body-weight in normal and in anuric rabbits whose ureters had been tied 1—48 and 72 hours respectively before the injection. I—at least three criteria of pulmonary oedema. S=spontaneous death

	Lung weight g/kg body weight	Wet weight mg by gross haemorrhage	Pulmonary oedema 1 ml by gross haemorrhage	Freddy 1 ml by gross haemorrhage	Alveolar oedema	Lung changes	X-ray cardiac enlargement	Pleural effusion ml	Aspirate ml	V.P.V. mg per 100 ml	Length of arterial adrenaline injection
I Adrenaline in normal rabbits											
L 10.51		88.7	++	++	+	++	++	0	12	85	100 S
L 12.0		88.0	++	++	+	0	0	0	2	68	8 S
L 10.28		87.0	++	++	++	++	?	0	0	44	13 S
L 9.78		86.3	++	++	++	++	++	0	0	—	35 S
L 8.10		83.6	++	+	++	++	++	4	0	70	310 S
L 6.96		78.7	++	0	+	0	0	0	0	58	325 S
L 5.11		81.5	+	0	+	0	+	0	11	78	185 S
L 5.01		80.0	0	0	—	0	+	0	0	50	3 S
L 4.14		81.7	0	0	—	0	+	3	2	42	320 S
L 4.11		81.7	+	0	—	0	+	1	0	34	3 S
Mean	8.20	83.8						1	3	80	
II Adrenaline 2—48 hours after ligation of ureters											
L 14.51		80.7	++	++	+	++	++	5	0	96	330 S
L 12.61		86.8	++	++	++	++	++	0	0	60	15 S
L 12.20		88.3	++	++	++	++	++	3	1	62	300 S
L 11.08		86.0	++	++	++	++	?	1	0	78	15 S
L 8.53		86.6	+	+	+	+	++	4	0	81	330 S
L 7.8		85.2	0	0	0	0	0	0	0	67	70 S
L 5.17		87.9	0	0	0	—	—	0	0	60	10 S
L 3.78		81.7	0	0	—	0	0	0	0	65	316 S
L 3.77		81.0	0	0	—	0	0	0	0	60	305 S
L 3.76		78.5	0	0	—	0	0	0	0	97	330 S
Mean	8.08	81.8						1	0	72	

[illegible]

Table 22. Results after intramuscular injection of adrenaline 1 mg per kg body weight in normal and in anuric rabbits whose ureters had been tied 1-4, 48 and 72 hours respectively before the injection. L=at least three criteria of pulmonary oedema S=spontaneous death

Rabbit	L	Lung weight g/kg body weight	Wet weight of lung g	Pulmonary oedema by gross observation	Alveolar oedema	Lung haemages	X-ray Canalic enlarge- ment	Pleural fl id ml	Anethes ml	N P V mg per 100 ml	Length of earr   after adrenalin in section
Adrenaline in normal	L	10.51	88.7	++	+	++	++	0	12	85	160
	L	12.70	88.0	++	+	++	0	0	2	08	8
	L	10.28	87.0	++	+	++	?	0	0	44	13
	L	9.78	86.2	++	+	++	++	0	0	—	50
	L	8.10	83.8	++	+	++	++	1	0	76	310
	L	0.26	78.7	+	+	++	0	0	0	68	325
	L	5.11	81.8	+	+	0	+	0	11	78	185
	L	5.01	80.9	0	—	0	+	0	0	50	3
	L	4.11	81.7	0	—	0	+	2	2	42	370
	L	1.11	81.7	+	—	0	+	1	0	31	3
Mean		8.20	83.8					1	3	60	
Adrenaline 1-4 hours	L	14.51	80.7	++	+	++	?	5	0	06	330
	L	12.01	86.8	++	+	++	++	0	0	00	15
	L	12.20	88.3	++	+	++	++	2	1	03	300
	L	11.08	86.9	++	+	++	?	1	0	70	15
	L	8.53	86.6	+	+	++	++	1	0	81	330
	L	5.28	82.2	0	0	0	0	0	0	07	10
	L	5.17	82.9	0	0	—	—	0	0	60	10
	L	3.78	81.7	0	—	0	0	0	0	35	315
	L	3.77	81.0	0	—	0	0	0	0	00	305
	L	3.0	85	0	—	0	0	0	0	07	330
Mean		8.08	81.8					1	0	72	





Table 23. Results after infusion of Ringer's solution 15% of the body-weight for 1 1/2 hours and injection of adrenaline intramuscularly 1 mg per kg body-weight in rabbits whose ureters had been tied 3-4, 48 and 72 hours respectively before the infection. *Least* at least three criteria of pulmonary oedema S=spontaneous death

Long weight g/kg body weight	Wter weight g/kg body weight	Pulmonary oedema by gross inspection	Prodney fl fibre	Al colic oedema	I ng lms gss	X ray Canila ent re- ment	Pleural fl d wt	Aeriles ml	\ P \ g per 100 g	Length survival adrenaline injection min	S
L 10.97	87.7	++	++	+	++	+	10	12	75	325	
L 19.79	90.3	++	++	+	++	++	98	38	80	110	
L 8.85	87.5	++	++	+	++	++	33	68	82	315	
L 7.77	84.2	++	++	+	++	++	2	12	80	00	
L 0.55	85.9	+	++	+	++	+	13	48	75	65	
L 5.97	85.0	0	0	—	0	+	3	10	00	300	
L 5.77	85.7	+	0	+	+	+	0	35	75	750	
L 5.58	85.0	0	0	—	0	+	10	11	60	330	
L 5.11	81.1	0	0	—	0	0	25	80	00	335	
L 5.03	83.3	0	0	—	0	+	13	58	67	300	
Mean 7.25	85.5						10	38	71		
L 10.72	85.9	++	++	0	++	+	1	30	108	15	
L 13.18	88.1	++	++	0	++	+	2	58	180	5	
L 13.00	87.1	++	++	0	++	+	2	23	175	20	
L 12.97	87.8	++	++	0	++	+	2	4	107	15	
L 5.73	81.5	0	0	—	+	++	7	35	108	315	
L 5.18	83.5	0	0	—	0	++	4	115	201	320	
L 5.37	83.0	0	0	—	0	++	8	38	172	300	
L 5.03	83.0	+	0	0	0	++	1	75	105	115	
L 1.77	82.0	0	0	0	0	++	8	18	162	330	
L 1.40	80.8	0	0	0	0	++	20	51	220	310	
Mean 9.02	85.9						6	45	182		

30 animals but the number was not sufficient in 1 non-overhydrated and 8 overhydrated animals. Four animals in which pulmonary oedema was established by at least three other criteria had normal water content. One rabbit had a water content of only 78.7 % but the lung weight, gross findings (numerous small haemorrhages in the lung parenchyma) and microscopical picture established the diagnosis of pulmonary oedema.

Microscopical alveolar oedema was present in 30 out of 46 animals, including 28 which had at least two other criteria of pulmonary oedema. Five overhydrated animals with pulmonary oedema by four or five other criteria had no alveolar oedema (similar observations have been described earlier by Viascher et al.)

In 24 rabbits microscopical examination was not performed, as autopsy showed pale-red air-filled lungs with out frothy fluid and of normal weight. Their lung weights had been calculated per kg body weight on the day of injection of adrenaline. After the experiments, however when calculated per kg body weight on the day of ureteral ligation, the lung weights were higher than normal in 2 of these cases.

The commonest microscopical findings in the rabbits with pulmonary oedema evidenced by at least three criteria were clearly visible alveolar oedema, marked hyperaemia (mentioned earlier by Poulsen [21]); moderate amounts of red cells in the alveoli, an increase in the numbers of alveolar phagocytes, and perilarterial interstitial oedema.

There were many exceptions, possibly attributable to overhydration and the extent of the oedema. In the series of non-overhydrated animals with pulmonary oedema evidenced by at least three criteria alveolar oedema graded as +++ was noted in 8 cases, ++ in 5, and + in 7 cases. In the series of overhydrated animals with pulmonary oedema by at least three criteria, alveolar oedema +++ was present in none, ++ in 1 case, + in 9 cases. In 5 there was no alveolar oedema and in 1 case the preparation was lost.

The investigation shows that the frequency of distinct alveolar oedema was much lower in overhydrated than in non-overhydrated rabbits. In cases of slight pulmonary oedema the alveolar oedema was often more distinct and more often seen occurring in patches than in cases of severe pulmonary oedema in which it could be indistinct and continuous, and in which the whole microscopical picture looked almost normal.

Perilarterial interstitial oedema was not seen in every rabbit with pulmonary oedema. In those that had red dots on the lung surfaces focal haemorrhages were seen microscopically. Bluish-pink lungs were slightly atelectatic. Nor was there any thickening of the alveolar wall in lungs with a high water content. Characteristic of the whole series was that bronchial changes were absent, atelectatic areas uncommon, and haemorrhages rare. Leucocytes were seen diffusely in increased numbers in scarcely half the cases. Foci of infection were not seen.

monary oedema) and 2 had markedly enlarged heart (2 had pulmonary oedema). Thus there was no high degree of correlation between heart size and pulmonary changes.

**Radiographic changes** With only one exception all the examined animals (63 out of 70) had radiographically normal lungs immediately before the adrenaline injection. This one rabbit which was overhydrated developed highly frothy pulmonary oedema immediately after the injection. After the adrenaline injection 32 animals (46 %) developed pulmonary changes and in 30 (51 %) X ray of the lungs was normal the picture could not be assessed in the remaining 2 cases (3 %).

The result of the radiographic examination showed good agreement with other criteria of lung oedema (Tables 22 and 23). A noteworthy exception was a rabbit in which the lungs were normal on X ray at the moment of death—8 minutes after the adrenaline injection—although frothy fluid was gushing forth from its nose and mouth at autopsy 5 minutes later the animal was found to have pulmonary oedema by all postmortem criteria. In another animal the lungs *in vivo* showed moderate radiographic changes which could be interpreted as oedema; autopsy a few minutes later revealed extensive bilateral atelectasis and only sparse pleural and ascitic fluid.

#### *Postmortem findings*

Gross observation at autopsy showed evidence of pulmonary oedema in 37 out of 70 rabbits. In 30 cases there was

distinct evidence in the form of reddish pink blotches or spots over the lung surfaces and profuse flow of fluid on section of the lung. In the other 7 rabbits there was slight evidence of pulmonary oedema namely numerous red dots changing into small red patches in the hilar region. Both large and small lesions showed a clear tendency to accumulate at the hilus and the base. In 9 rabbits a few red dots were seen on the lung surfaces and 5 had small bluish pink lungs.

In 34 of the above mentioned 37 rabbits the macroscopical diagnosis of pulmonary oedema was supported by at least two other criteria. One rabbit had no gross evidence of lung oedema of the type discussed here although three other criteria supported the diagnosis.

Frothy fluid in the air ways was found in 27 animals, all of which had pulmonary oedema evidenced by at least four criteria. In 7 animals with pulmonary oedema by at least three criteria there was no frothy fluid in the air ways.

The lung weight in 30 of 70 animals exceeded the upper limit of normal range. The diagnosis of pulmonary oedema was supported by at least two other criteria in 34 of these 30 (Tables 22 and 23).

The water content of the lungs exceeded the upper limit of normal range in 50 % (30 of 70 animals) the figure for the overhydrated rabbits was 71 % and for the non-overhydrated one 40 % (Tables 22 and 23). An adequate number of criteria for the diagnosis of pulmonary oedema were fulfilled in

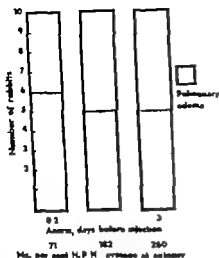


Diagram 11 Frequency of pulmonary oedema after intravenous infusion of Ringer's solution, 15 % of the body-weight for 1 1/2 hours, and intramuscular injection of adrenaline, 1 mg per kg body-weight, in rabbits which had been anuric for 0.2, 2, and 3 days, respectively before the injection.

of adrenaline in 6 of 10 controls, in 5 of 10 rabbits with anuria for 1—4 hours, in 4 of 10 rabbits with anuria for 48 hours, and in 3 of 10 rabbits with anuria for 72 hours.

Overhydrated rabbits (Table 23 and Diagram 11) Pulmonary oedema as defined here occurred after injection of adrenaline in 6 of 10 animals with anuria for 1—4 hours, in 5 of 10 with anuria for 48 hours, and in 5 of 10 with anuria for 72 hours.

Thus, the frequency of adrenaline-induced pulmonary oedema in the rabbits whose ureters had been ligated decreased slightly with increasing uraemia, but the differences between slightly uraemic and highly uraemic animals is not statistically significant

in either the overhydrated or the non-overhydrated series.

### *Pulmonary oedema and overhydration*

The frequency of pulmonary oedema was higher in overhydrated than in non-overhydrated animals, but the difference is not significant and it was difficult to render the two groups comparable. The adrenaline concentration in blood and tissues can have differed between the two groups because of a larger fluid space ( $> 15\%$ ) and a higher dose (16 %) in the overhydrated animals, different rate of absorption for the adrenaline injected intramuscularly differences in circulatory rate, and other factors.

### *Survival time in relation to frequency of pulmonary oedema and degree of uraemia*

Among the 70 rabbits in the series, 7 died within 10 minutes of the injection of adrenaline 3 of these 7 had pulmonary oedema according to the definition used here (43 %) 22 died spontaneously within 11—60 minutes of the injection 15 of these had pulmonary oedema (68 %) 14 died spontaneously within 61—310 minutes of the injection 6 of these had pulmonary oedema (43 %) 27 rabbits were bled within 5 hours of the injection 10 of these had pulmonary oedema (87 %) Cases of slight transient pulmonary oedema can of course have been missed and left out of the records.

Among those that died spontaneously without pulmonary oedema, 11 had been anuric for 72 hours, 3 for 48

### Criteria of pulmonary oedema

The diagnosis of pulmonary oedema following injection of adrenaline in uraemic rabbits (Diagram 18 Tables 23 and 24) could in most cases be based on the correlation obtained between the methods of examination. The diagnosis was in 19 cases based on six positive criteria in 10 cases on five, in 8 cases on four and in 2 cases on three criteria. One X ray examination and one microscopical examination failed.

In 36 cases the number of positive criteria was less than three, and it was therefore considered that pulmonary oedema was not present. In 23 of these cases there were no positive criteria in 8 there was one criterion and in 5 there were two criteria. One X ray examination failed.

In 23 cases the lungs were not examined microscopically. In 16 of them there were no criteria in 5 there was one, and in 2 there were two criteria of slight pulmonary oedema (in the series of overhydrated animals with anuria for 3—4 hours). If alveolar oedema had been found in these 2 cases the number of criteria would thus have been sufficient to establish the diagnosis of pulmonary oedema. This, however, would have had very little influence on the conclusions concerning the relationship between the frequency of pulmonary oedema and the degree of uraemia on one hand and overhydration on the other.

*Atelectasis, bleedings, and pneumonia* were seldom seen. In 4 overhydrated rabbits alveolar oedema was absent although they had severe pulmonary

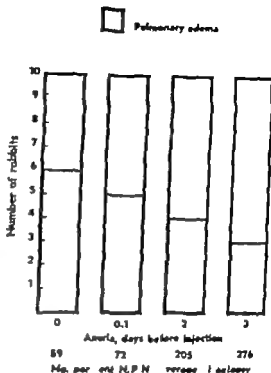


Diagram 10. Occurrence of pulmonary oedema after intramuscular injection of epinephrine 1 mg per kg body weight, in normal rabbits and rabbits, which had been anuric for 0, 1, 2, and 3 days, respectively before the injection.

oedema evidenced by all the other criteria. Similar observations have been made earlier by, for instance, Visscher et al. Four overhydrated rabbits with pulmonary oedema by at least four criteria had no frothy fluid in the airways; profuse amounts of secretion were seen, however. The differential diagnosis was difficult in these cases.

### Pulmonary oedema and degree of uraemia

The control series and the non overhydrated anuric rabbits (Table 22 and Diagram 10). Pulmonary oedema as defined here occurred after injection

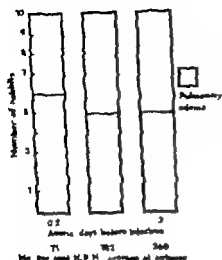


Diagram 11 Frequency of pulmonary oedema after intravenous infusion of Ringer's solution, 15 % of the body-weight for 1 1/2 hours, and intramuscular injection of adrenaline, 1 mg per kg body-weight, in rabbits which had been anuric for 0.2, 1.5, and 3 days, respectively before the injection.

of adrenaline in 6 of 10 controls, in 5 of 10 rabbits with anuria for 1—2 hours, in 4 of 10 rabbits with anuria for 48 hours, and in 3 of 10 rabbits with anuria for 72 hours.

Overhydrated rabbits (Table 23 and Diagram 11) Pulmonary oedema as defined here occurred after injection of adrenaline in 6 of 10 animals with anuria for 1—4 hours, in 5 of 10 with anuria for 48 hours, and in 5 of 10 with anuria for 72 hours.

Thus, the frequency of adrenaline induced pulmonary oedema in the rabbits whose ureters had been ligated decreased slightly with increasing uraemia, but the differences between slightly uraemic and highly uraemic animals is not statistically significant

in either the overhydrated or the non-overhydrated series.

### *Pulmonary oedema and overhydration*

The frequency of pulmonary oedema was higher in overhydrated than in non-overhydrated animals, but the difference is not significant and it was difficult to render the two groups comparable. The adrenaline concentration in blood and tissues can have differed between the two groups because of a larger fluid space ( $> 15\%$ ) and a higher dose (15 %) in the overhydrated animals, different rate of absorption for the adrenaline injected intramuscularly differences in circulatory rate, and other factors.

### *Survival time in relation to frequency of pulmonary oedema and degree of uraemia*

Among the 70 rabbits in the series, 7 died within 10 minutes of the injection of adrenaline 3 of these 7 had pulmonary oedema according to the definition used here (43 %) 22 died spontaneously within 11—60 minutes of the injection 15 of these had pulmonary oedema (68 %) 14 died spontaneously within 61—310 minutes of the injection 8 of these had pulmonary oedema (43 %) 27 rabbits were bled within 5 hours of the injection 10 of these had pulmonary oedema (37 %) Cases of slight transient pulmonary oedema can of course have been missed and left out of the records.

Among those that died spontaneously without pulmonary oedema 11 had been anuric for 72 hours, 3 for 48

### Criteria of pulmonary oedema

The diagnosis of pulmonary oedema following injection of adrenaline in uraemic rabbits (Diagram 18 Tables 23 and 24) could in most cases be based on the correlation obtained between the methods of examination. The diagnosis was in 10 cases based on six positive criteria, in 10 cases on five in 3 cases on four and in 2 cases on three criteria. One X ray examination and one microscopical examination failed.

In 36 cases the number of positive criteria was less than three, and it was therefore considered that pulmonary oedema was not present. In 23 of these cases there were no positive criteria in 8 there was one criterion and in 5 there were two criteria. One X ray examination failed.

In 23 cases the lungs were not examined microscopically. In 16 of them there were no criteria in 5 there was one, and in 2 there were two criteria of slight pulmonary oedema (in the series of overhydrated animals with anuria for 3—4 hours). If alveolar oedema had been found in these 2 cases the number of criteria would thus have been sufficient to establish the diagnosis of pulmonary oedema. This, however, would have had very little influence on the conclusions concerning the relationship between the frequency of pulmonary oedema and the degree of uraemia on one hand and overhydration on the other.

*Atelectasis, bleedings, and pneumonia* were seldom seen. In 4 overhydrated rabbits alveolar oedema was absent although they had severe pulmonary

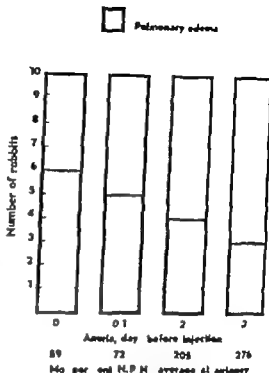


Diagram 10 Occurrence of pulmonary oedema after intramuscular injection of epinephrine 1 mg per kg body weight, in normal rabbits and rabbits, which had been anuric for 0.1, 2, and 3 days, respectively before the injection.

oedema evidenced by all the other criteria. Similar observations have been made earlier by, for instance, Visscher et al. Four overhydrated rabbits with pulmonary oedema by at least four criteria had no frothy fluid in the airways; profuse amounts of secretion were seen, however. The differential diagnosis was difficult in these cases.

### Pulmonary oedema and degree of uraemia

The control series and the non-overhydrated anuric rabbits (Table 22 and Diagram 10) pulmonary oedema as defined here occurred after injection

## Bilateral vagotomy

Many surveys of the extensive literature on vagotomy have been presented (e.g. 90, 158, 168, 214, 225, 246). Most of the experiments were carried out in rabbits, rats, and guinea pigs. In dogs and cats, on the contrary, vagotomy permits indefinite survival (214). The following short survey of the literature will serve only as a background to my own experiments reported in this section. The account refers to vagotomy without previous overhydration and vagotomy in overhydrated animals.

a) **Vagotomy.** After bilateral vagotomy in experimental animals, Lower (1664) noted death from dyspnoea. Vieusseux (1718) accumulations of blood in the lungs, and Valzilha (1746) blood stained froth in the mouth. Haller (1746—1760) ascribed the pulmonary changes to aspiration of gastric contents. Legallois (1812) suggested that the cause of death would be asphyxia due to reduced glottis, hyperaemia in the lungs, and/ or the presence of serous fluid in the bronchi. Traube (1846) showed that tracheotomy protected rabbits against agotomy oedema, concluding that vocal-cord paralysis was the mechanism of the lung lesion. Schiff (1850) in contradiction of Traube, found that pulmonary oedema occurred even after tracheotomy and did not note any lung lesions after bilateral division of the recurrent

laryngeal nerve. Schiff formed the hypothesis of pulmonary vasomotor paralysis whereas Claude Bernard (1858) ascribed the lesions to altered respiratory dynamics. Löwit (1893) did not find any rise in left atrial pressure and concluded that the lung oedema was brought about by vasodilatation with increased accumulation of blood and increased vascular permeability. Shafer (1920) noted pulmonary oedema in association with slow asphyxia without agotomy and suggested that this was the causative factor in the lung oedema following vagotomy. Farber (1937) found that tracheotomy and artificial respiration did not protect rabbits against vagotomy oedema. On direct inspection he noted that the lung lesion began as petechia which gradually fused into large areas showing red discoloration which at autopsy were found to be consolidated areas. He considered that vagal denervation increased the permeability of the lung capillaries. Lother (1939) found that with careful bronchial toilet after vagotomy outspoken lung oedema did not occur. He suggested that circulatory failure in association with anoxia could be a factor involved in agotomy oedema.

Short (1941) showed that tracheotomy alone without vagotomy can give rise to stagnation of secretion in the bronchi and cause slight pulmonary oedema. Reichman (1946) found that the inspiratory phase was prolonged after vagotomy and that even with other techniques impeded expiration can cause pulmonary oedema. This was confirmed in experiments by



hours, and 2 for a few hours, while 3 belonged to the control group

Thus, among highly uraemic animals overhydrated as well as non overhydrated ones the number of those that died spontaneously without pulmonary oedema was greater than it was among slightly uraemic animals. The explanation could be that in high

ly uraemic animals death is more often due to some adrenaline effect other than pulmonary oedema. In interpreting the results of the adrenaline series it is therefore difficult to determine whether highly uraemic animals have an increased tendency toward pulmonary oedema.

Survey See page 144

## Bilateral vagotomy

Many surveys of the extensive literature on vagotomy have been presented (e.g. 90 158, 168 214 225 246). Most of the experiments were carried out in rabbits, rats, and guinea pigs. In dogs and cats on the contrary vagotomy permits indefinite survival (214). The following short survey of the literature will serve only as a background to my own experiments reported in this section. The account refers to vagotomy without previous overhydration and vagotomy in overhydrated animals.

a) *Vagotomy* After bilateral vagotomy in experimental animals, Lower (1864) noted death from dyspnoea, Vlemmings (1715) accumulations of blood in the lungs, and Valsalva (1740) blood-stained sputum in the mouth. Haller (1756—1760) ascribed the pulmonary changes to aspiration of gastric contents. Legallois (1812) suggested that the cause of death would be asphyxia due to a reduced glottis, hyperaemia in the lungs, and/or the presence of serous fluid in the bronchi. Traube (1816) showed that tracheotomy protected rabbits against vagotomy oedema, concluding that local cord paralysis was the mechanism of the lung lesion. Schiff (1830) in contradiction of Traube, found that pulmonary oedema occurred even after tracheotomy and did not note any lung lesion after bilateral division of the recurrent

laryngeal nerve. Schiff formed the hypothesis of pulmonary vasomotor paralysis whereas Claude Bernard (1858) ascribed the lesions to altered respiratory dynamics. Löwit (1893) did not find any rise in left atrial pressure and concluded that the lung oedema was brought about by vasodilatation with increased accumulation of blood and increased vascular permeability. Shafer (1920) noted pulmonary oedema in association with slow asphyxia without vagotomy and suggested that this was the causative factor in the lung oedema following vagotomy. Farber (1937) found that tracheotomy and artificial respiration did not protect rabbits against vagotomy oedema. On direct inspection he noted that the lung lesions began as petechia which gradually fused into large areas showing red discoloration which in autopsy were found to be consolidated areas. He considered that vagal denervation increased the permeability of the lung capillaries. Lorber (1939) found that with careful bronchial toilet after vagotomy outspoken lung oedema did not occur. He suggested that circulatory failure in association with anaemia could be a factor involved in vagotomy oedema.

Short (1914) showed that tracheotomy alone without vagotomy can give rise to stagnation of secretion in the bronchi and cause slight pulmonary oedema. Retckman (1946) found that the inspiratory phase was prolonged after vagotomy and that even with other techniques impeded inspiration can cause pulmonary oedema. This was confirmed in experiments by

Baruch (1935) who found that oedema developed in the hilar and the basal regions in dogs submitted to slight inspiratory resistance to breathing for 6 hours Reichsman found as did Farber (1940) that the blood and plasma volumes decreased greatly after vagotomy Haddy et al (1950) noted that the pulmonary vein pressure rose as a result of inspiratory airway resistance. Keyrillinen & Paasonen (1958) showed that ether anaesthesia at vagotomy in rats reduced the survival time in comparison with chloral, urethane, and sodium pentobarbitone anaesthesia, that atropine in high doses reduced the degree of pulmonary oedema that sex and age was of no significance in the formation of pulmonary oedema and adrenalectomy had no effect on its development.

b) *Vagotomy—overhydration.* Kraus (1913) found that rapid intravenous infusion of saline, about 400 ml before and 50 ml after the vagotomy produced frothy pulmonary oedema in rabbits If adrenalin was given in addition the oedema was aggravated Kraus considered that the lung oedema was due to loss of innervation of the lung vessels. Brunn (1913) reported that tracheotomized vagotomized rabbits developed pulmonary oedema less readily than did non tracheotomized rabbits after the same amount of saline. If the rabbits were nephrectomized bilaterally before the vagotomy and the infusion, pulmonary oedema did not occur Farber (1940) however noted no difference in the frequency of pulmonary oedema (gross observations) in association with overhydration between tracheotomized and non tracheotomized vagotomized rabbits. After unilateral vagotomy pulmonary oedema rarely occurred in the rabbits, despite infusion of saline at a rate of 40 ml per minute the total amount being 250–400 ml Reichsman (1946) showed that pulmonary oedema could be precipitated regularly by means of rapid intravenous infusion of saline in rats submitted to inspiratory resistance to breathing Unilateral or bi

lateral sympathectomy reduced the tendency toward pulmonary oedema following massive infusions of saline in vagotomized dogs (52)

### *Own investigations*

The purpose of this series was to study the frequency of pulmonary oedema at different degrees of uraemia in non overhydrated and overhydrated vagotomized rabbits whose ureters had been tied bilaterally

All the animals were first submitted to bilateral ureteral ligation under local anaesthesia. In the overhydrated series the animals were given fluid before they underwent vagotomy Bilateral vagotomy 0–48 hours after the ureteral ligation was performed under local anaesthesia through a median incision in the neck.

29 non-overhydrated anuric rabbits were divided into two series which were vagotomized a) 0–4 hours (15 animals) and b) 48 hours (14 animals) after the ureteral ligation

28 overhydrated anuric rabbits were divided into two series which were vagotomized a) 3–5 hours (14 animals) and b) 48 hours (14 animals) after the ureteral ligation. Two hours before the vagotomy Ringer's solution was infused intravenously for 1½ hours in amounts equalling 15 % of the body weights

Two rabbits which died from suffocation during the operation were excluded from the series 2 rabbits which died within 10 minutes of the vagotomy are included Three rabbits which received infusion after the vagotomy were markedly distressed by even the

lightest possible fixation and died from suffocation during the infusion.

Respiratory rate measurements and electrocardiograms were taken before the ureteral ligation, before and after the vagotomy and before the blood letting whereas chest X ray could not be performed as planned, because the animals had attacks of suffocation when being fixed on the table. Four of those that were examined first died during the examination. Radiographs were therefore taken only before the bloodletting or within 5 minutes of spontaneous death. In view of the risk of suffocation, the animals were allowed to move freely in the room after the vagotomy.

The rabbits that did not die spontaneously were bled within 14 1/2 hours of the vagotomy. Four moribund rabbits were bled within 5—10 hours. In spite of frequent checkings even during the night, it was difficult to estimate the probable survival time, that is, the suitable time for bloodletting before the planned time. Four rabbits were excluded from the series, as rigor mortis had already set in when death was noted.

#### *Survival time*

The series of non-overhydrated anuric rabbits a) Among 16 animals which had been anuric for 0—4 hours before the vagotomy none died during the first 7 hours. 2 died spontaneously within 11 hours. 13 were bled within about 14 1/2 hours. b) Out of 17 animals which were vagotomized after 48 hours of anuria, 4 died spontaneously within the first 7 hours and an

other 5 within about 14 1/2 hours. 4 were bled in moribund states within 5—10 hours of the vagotomy and 4 survived long enough to be bled within about 14 1/2 hours.

The series of overhydrated anuric rabbits a) Out of 15 animals which had been anuric for 3—5 hours, 4 died spontaneously within the first 7 hours and another 5 within 14 hours of the vagotomy. 5 were bled within about 14 1/2 hours. b) Out of 14 animals which were vagotomized after 48 hours of anuria, 7 died spontaneously within the first 7 hours and a further 3 within 14 hours. 4 were bled within about 14 1/2 hours.

In these groups the average survival time was lowest for overhydrated animals with anuria for 48 hours (8.2 hours) and highest for non-overhydrated animals with anuria for 0—4 hours (13.8 hours).

#### *Symptoms in vivo*

Under this heading a short account will be given of the generalized symptoms that were noted in vivo after vagotomy. It will be seen from the account of the postmortem findings under the next heading that the diagnosis of lung oedema by the criteria that were well applicable in the previous series offered great difficulties in some of the vagotomy cases. Only after a detailed discussion of the sources of error in the different criteria under the experimental conditions used here, will it be possible to discuss the relation between pulmonary oedema, diagnosed with varying degrees of reliability

and symptoms in vivo This will be done under a separate heading below

**Appearance, behaviour and respiration** As is usual vagotomy was regularly followed by bradypnoea In about two-thirds of the animals respiration became piping and rattling The respiratory difficulties increased on slightest exertion and particularly on attempts at holding the animals fast Stridulous breathing was commoner in overhydrated than in non-overhydrated animals The general condition was relatively little affected by the vagotomy

The respiratory rate fell in the whole series from an average of 137 (60—202) per minute before the vagotomy to 51 (24—84) per minute after the operation.

**E.C.G. and heart rate** The heart rate increased from an average of 230 (174—316) beats per minute before the vagotomy to 263 (180—340) beats per minute immediately after the vagotomy

Among 31 rabbits with anuria for 48 hours E.C.G. before vagotomy showed no abnormalities in 20 absence of T waves in 8 negative T waves in 2 and a deformed QRS-complex in 1 A few minutes after the vagotomy the E.C.G. was normal in 10 of these rabbits the T wave absent in 2 and negative in 8 and the QRS-complex deformed in 11 Out of 30 animals with anuria for only a few hours 2 had slight E.C.G. changes after the vagotomy

The heart shadow increased within a few minutes of the vagotomy in 6 of 10 examined non-overhydrated ani-

mals Subsequent assessment was difficult because of pulmonary changes (Tables 24 and 25)

Chest X ray showed no abnormalities in 10 cases examined before and immediately after the vagotomy

#### *Radiographic pulmonary changes postmortem findings and pulmonary oedema*

As already mentioned the diagnosis of pulmonary oedema in the vagotomy series offered great difficulties in some cases the uncertainty attached to each criterion will be discussed in the following It will be seen from Tables 24 and 25 that this refers only to some of the 57 animals, 25 rabbits (44 %) had, at most, two of six criteria of pulmonary oedema and hence pulmonary oedema was regarded as not present. In 18 cases (31 %) the presence of pulmonary oedema was very probable 11 rabbits had all the six criteria and 7 had five criteria In 3 rabbits with four criteria (lung weights 19.02 g 11.12 g and 9.58 g) but without or with insignificant pneumonia microscopically X ray was not performed but would probably have supported the diagnosis of pulmonary oedema In the remaining 14 (25 %) the presence of pulmonary oedema was more difficult to establish or to exclude

As has been mentioned earlier X ray evidence of unilateral or apical changes was seen in 11 of 31 animals with lung lesions The most frequent microscopical finding in these 11 cases was pneumonia with atelectatic and

No.	Sex	Age (yr)	Weight (kg)	Landed by observer	In the airway	Changes	Number seen	Regulatory hours	S	P	P	P
1	L	11.34	83.6	+	+	+	7	87	18.4	S		
2	L	7.86	87.7	+	+	+	+	80	7.0	S		
3	L	7.88	81.9	+	+	+	+	115	14.7	P		
4		6.31	83.4	+	+	+	0	107	14.5	P		
5		5.65	82.9	+	+	+	0	110	14.5	P		
6		5.07	81.1	+	+	+	0	124	14.7	P		
7		4.97	79.1	+	+	+	0	79	14.6	P		
8		4.82	77.9	+	+	+	0	90	14.5	P		
9		4.71	82.3	+	+	+	0	86	14.5	P		
10		4.68	81.7	+	+	+	0	93	14.5	P		
11		4.58	81.8	+	+	+	0	122	14.5	P		
12		4.49	82.3	+	+	+	0	110	14.4	P		
13		4.46	81.8	+	+	+	0	108	14.3	P		
14		4.33	82.3	+	+	+	0	96	14.6	P		
15		4.23	82.8	+	+	+	0	121	14.5	P		
		5.63	83.6	+	+	+	0	162				
16	L	18.42	80.9	+	+	+	7	—	8.9	S	P	
17	L	16.93	82.3	+	+	+	7	375	7.0	S	P	
18	L	16.58	80.3	+	+	+	+	—	22	S	P	
19	L	8.07	81.9	+	+	+	7	253	8.0	S	P	
20	L	8.58	82.4	+	+	+	7	498	14.3	S	P	
21	L	8.50	81.8	+	+	+	7	291	7.9	S	P	
22		8.18	82.3	+	+	+	7	225	14.0	S		
23		5.45	82.7	+	+	+	0	258	8.1	S		
24		4.91	81.7	+	+	+	0	218	10.2	S		
25		4.78	80.9	+	+	+	0	279	14.3	S		
26		4.51	83.0	+	+	+	0	228	14.3	S		
27		4.33	81.9	+	+	+	0	218	14.5	S		
28		3.78	82.5	+	+	+	11	170	0.0	S		
29		3.63	80.3	+	+	+	0	227	14.8	S		
		7.35	83.8	+	+	+	0	241				

11/2/88

L. V. Gokany 48 hours after ligation

11/2/88  
L. V. Gokany 48 hours after ligation

Table 25 Results after Infusion of Ringer's solution, 15% of the body-weight for 1½ hours and bilateral vagotomy in anuric rabbits whose ureters had been tied 3-5 and 48 hours respectively before the vagotomy  
L = at least three criteria of pulmonary oedema. P = microscopic evidence of severe pneumonia. S = spontaneous death

	U biat No.	Lw g weight g/g body weight	Wt cent l 1 h f	Pulmonary oedema by gross section at 1 h	Trothy fl id 1 the	Al solar oedema	Lung has enlargement	X ray Cardiac enlargement	Pleural fl ml ml	Axilles ml	N r K mg per 100 ml	Length of survival after	
L, 1-5 hours after ligation	20	L	10.02	100.0	+	+	0	+	+	+	100	13.5	S
	21	L	12.01	87.1	+	+	+	+	+	+	103	13.9	S
	22	L	12.82	87.1	+	+	+	+	+	+	110	13.1	S
	23	L	11.72	87.7	+	+	+	+	+	+	100	11.4	S
	24	L	10.42	89.4	+	+	+	+	+	+	103	14.5	S
	25	L	9.58	87.3	+	+	0	+	+	+	103	14.5	S
	26	L	9.18	85.7	+	+	0	+	+	+	103	14.5	S
	27	L	7.80	83.7	+	+	0	+	+	+	103	14.5	S
	28	L	7.09	86.1	+	+	0	+	+	+	103	14.5	S
	29	L	7.05	81.0	0	0	0	+	+	+	103	14.5	S
L, 3-5 hours after ligation	30	L	5.17	82.5	0	0	0	+	+	+	103	14.5	S
	31	L	5.38	81.7	0	0	0	+	+	+	103	14.5	S
	32	L	4.89	81.7	+	+	0	+	+	+	103	14.5	S
	33	L	4.56	83.0	0	0	0	+	+	+	103	14.5	S
	34	L	4.14	81.9	0	0	0	+	+	+	103	14.5	S
	35	L	4.14	81.9	0	0	0	+	+	+	103	14.5	S
	36	L	4.14	81.9	0	0	0	+	+	+	103	14.5	S
	37	L	4.14	81.9	0	0	0	+	+	+	103	14.5	S
	38	L	4.14	81.9	0	0	0	+	+	+	103	14.5	S
	39	L	4.14	81.9	0	0	0	+	+	+	103	14.5	S
L, 15 hours after ligation	40	L	11.72	90.2	+	+	0	+	+	+	103	14.5	S
	41	L	12.01	90.4	+	+	+	+	+	+	103	14.5	S
	42	L	12.11	80.5	+	+	+	+	+	+	103	14.5	S
	43	L	11.29	81.1	+	+	+	+	+	+	103	14.5	S
	44	L	11.12	83.1	+	+	+	+	+	+	103	14.5	S
	45	L	7.37	81.9	+	+	0	+	+	+	103	14.5	S
	46	L	7.13	83.9	+	+	0	+	+	+	103	14.5	S
	47	L	6.12	83.2	+	+	0	+	+	+	103	14.5	S
	48	L	5.96	82.1	+	+	0	+	+	+	103	14.5	S
	49	L	5.57	81.7	+	+	0	+	+	+	103	14.5	S
L, 48 hours after ligation	50	L	1.40	82.1	+	+	0	+	+	+	103	14.5	S
	51	L	1.67	82.0	+	+	0	+	+	+	103	14.5	S
	52	L	1.21	81.1	+	+	0	+	+	+	103	14.5	S
	53	L	1.21	81.1	+	+	0	+	+	+	103	14.5	S
	54	L	1.21	81.1	+	+	0	+	+	+	103	14.5	S
	55	L	1.21	81.1	+	+	0	+	+	+	103	14.5	S
	56	L	1.21	81.1	+	+	0	+	+	+	103	14.5	S
	57	L	1.21	81.1	+	+	0	+	+	+	103	14.5	S
	58	L	1.21	81.1	+	+	0	+	+	+	103	14.5	S
	59	L	1.21	81.1	+	+	0	+	+	+	103	14.5	S

haemorrhagic areas. None of these rabbits had a sufficient number of the criteria required for the diagnosis of pulmonary oedema in this series, that is, three criteria including increased water content or microscopical alveolar oedema (see the next heading).

Bilateral diffuse lesions in all the lobes were seen in 23 of the 34 cases. Radiographic pulmonary changes were seen in all animals that had sufficient criteria of pulmonary oedema. In 6 of the 23 animals with bilateral changes, the number of criteria required in this series was not sufficient, however—microscopically pneumonia, atelectasis, and/or haemorrhages were present.

Five rabbits, which at autopsy were found to have atelectasis as the main finding by gross and microscopical examination, normal lung weights, and more than 30 ml of pleural fluid (4 cases) had radiographic pulmonary changes which in 2 cases were bilateral and diffuse, in 2 cases unilateral and in 1 case localized in the upper lobes.

The radiologist has in 13 cases (see the sign ? in Tables 24 and 25) stated the presence of pulmonary processes other than oedema or both pulmonary oedema and some other process. None of these rabbits had microscopical evidence of pulmonary oedema alone.

Gross observation at autopsy. The average discharge of blood at the bloodletting was 49 (28—80) ml as against 64 (30—110) ml in the series of rabbits submitted to ureteral ligation without vagotomy. A reduced amount of blood after vagotomy was also noted by Reichsman and Farber

(84:214). The amounts of ascitic fluid and pleural fluid (Tables 24 and 25) differed insignificantly from those found in the animals of the series described in previous chapters.

Gross evidence that could indicate pulmonary oedema was present in 38 out of 57 rabbits. All the animals with pulmonary oedema evidenced by the criteria used in this series had such macroscopical changes. In 13 animals (Diagram 18) with such changes the diagnosis of pulmonary oedema could not be established by the criteria used in this series.

The macroscopical picture varied. Mottled or wholly dark red lung surfaces ( + + + ) were noted in 10 rabbits (the other criteria were also positive in 8; 1 criterion was lacking in 2). 10 rabbits had spotted, streaky or partly mottled lung surfaces ( + + ) but microscopical examination showed that one whitish-purple lung (no 42) was atelectatic hyperaemic and that in one brownish-pink spotted lung (no 17) there were large areas of pneumonia.

16 animals had changes graded as + a frequent finding in this group was brown, even and well-defined lesions which seemed to have arisen in the upper lobes. The observed changes ranged from single spots in the upper lobes to brown areas extending over the upper and middle lobes and the upper parts of the lower lobes. The rest of the lung surface was in some cases blue and pink as well, and in others also covered with red dots or with spots. Only those changes that extended beyond the upper lobes were



**Table 21** Results after infusion of Ringer's solution 15% of the body weight for 1½ hours and unilateral vagotomy in anuric rabbits whose ureters had been tied 3–5 and 48 hours respectively before the vagotomy. L, at least three criteria of pulmonary oedema; P, microscopical evidence of pulmonary oedema

[illegible]

haemorrhagic areas. None of these rabbits had a sufficient number of the criteria required for the diagnosis of pulmonary oedema in this series, that is, three criteria including increased water content or microscopical alveolar oedema (see the next heading).

Bilateral diffuse lesions in all the lobes were seen in 23 of the 34 cases. Radiographic pulmonary changes were seen in all animals that had sufficient criteria of pulmonary oedema. In 6 of the 23 animals with bilateral changes, the number of criteria required in this series was not sufficient however: microscopically pneumonia, atelectasis, and/or haemorrhages were present.

Five rabbits, which at autopsy were found to have atelectasis as the main finding by gross and microscopical examination, normal lung weights, and more than 30 ml of pleural fluid (4 cases) had radiographic pulmonary changes which in 2 cases were bilateral and diffuse, in 2 cases unilateral, and in 1 case localized in the upper lobes.

The radiologist has in 13 cases (see the sign 7" in Tables 24 and 25) stated the presence of pulmonary processes other than oedema, or both pulmonary oedema and some other process. None of these rabbits had microscopical evidence of pulmonary oedema alone.

Gross observation at autopsy. The average discharge of blood at the blood titing was 49 (28—80) ml as against 64 (30—110) ml in the series of rabbits submitted to ureteral ligation without vagotomy. A reduced amount of blood after vagotomy was also noted by Reichman and Farber

(81-214). The amounts of ascitic fluid and pleural fluid (Tables 24 and 25) differed insignificantly from those found in the animals of the series described in previous chapters.

Gross evidence that could indicate pulmonary oedema was present in 36 out of 57 rabbits. All the animals with pulmonary oedema evidenced by the criteria used in this series had such macroscopical changes. In 13 animals (Diagram 18) with such changes the diagnosis of pulmonary oedema could not be established by the criteria used in this series.

The macroscopical picture varied. Mottled or wholly dark red lung surfaces ( + + + ) were noted in 10 rabbits (the other criteria were also positive in 8, 1 criterion was lacking in 2). 10 rabbits had spotted, streaky or partly mottled lung surfaces ( + + ) but microscopical examination showed that one whitish purple lung (no 42) was atelectatic hyperaemic and that in one brownish pink spotted lung (no 17) there were large areas of pneumonia.

16 animals had changes graded as + a frequent finding in this group was brown, even and well-defined lesions which seemed to have arisen in the upper lobes. The observed changes ranged from single spots in the upper lobes to brown areas extending over the upper and middle lobes and the upper parts of the lower lobes. The rest of the lung surface was in some cases blue and pink as well, and in others also covered with red dots or with spots. Only those changes that extended beyond the upper lobes were

graded as "+". Throughout this group the diagnosis of pulmonary oedema was in doubt.

Frothy fluid in the air ways was noted in 10 of the 57 animals. Because of the profuse amounts of viscid mucus and fluid in the mouth nose and trachea in vagotomized rabbits, notably overhydrated ones, the diagnosis of pulmonary oedema by the presence of frothy fluid in the air passages was doubtful in this series. Phlegm or aspirated fluid can form froth and oedema fluid may contain very little air and resemble phlegm.

In 16 of the 10 animals with frothy fluid there were a sufficient number of the criteria of pulmonary oedema required in this series: frothy fluid was present in the majority of the rabbits that had the most distinct evidence of pulmonary oedema. Exceptions were 2 animals with seemingly considerable pulmonary oedema (lung weights 13.01 and 11.29 g per kg body weight; no pneumonia microscopically). Three rabbits (lung weights 6.21, 4.76 and 4.69 g) had frothy fluid in the trachea but bronchitis—bronchopneumonia were diagnosed microscopically and water contents were low: according to the criteria used in this series pulmonary oedema was regarded as not present.

Thus the presence of frothy fluid cannot be taken as conclusive evidence of pulmonary oedema in vagotomized animals.

The lung weight exceeded the upper limit of normal range in 31 out of 57 animals. 22 of these 31 rabbits had criteria of the kind required for the

diagnosis of pulmonary oedema in this series (see the following). Poulsen (206) stated that pneumonia and hyperaemia lead to increased lung weight. This may be the explanation of the increased lung weights in the 9 rabbits which did not have pulmonary oedema by the criteria used in this series: microscopically these 9 rabbits had pneumonia or hyperaemia but no alveolar oedema. This assumption is further supported by the data in Diagrams 14 and 16 in which the pneumonia cases from all the series and 3 rabbits without ureteral ligation have been brought together.

Under the experimental conditions of this study the lung weight is thus of definite value only in excluding higher degrees of pulmonary oedema and when pneumonia and hyperaemia can be ruled out.

The water content exceeded the upper limit of normal range in 21 out of the 57 animals (15 of these 21 were overhydrated). In 17 of the 21 animals with increased water contents the number of other criteria used in this series was sufficient to establish the diagnosis of pulmonary oedema (Tables 24 and 25). 4 animals had normal water contents, but pulmonary oedema by the criteria used in this series: they had gross evidence of pulmonary oedema, increased lung weights, and microscopical evidence of alveolar oedema but also appreciable haemorrhages (2 cases) or hyperaemia (2 cases). In 1 of the latter profuse stringy exudate was seen.

If the water content in the lung is only slightly increased in overhydrated

cases and if pulmonary changes other than oedema co-exist, the water content is of uncertain value in the diagnosis of pulmonary oedema.

Microscopical examination showed in the vagotomy series pneumonia, atelectasis, haemorrhage hyperaemia and/or oedema in varying combinations. When one process predominated, the microscopical diagnosis was easy but if several processes co-existed, evaluation became a problem. Was oedema secondary to haemorrhages or small pneumonic lesions, or were there both pulmonary oedema and pneumonia or pulmonary oedema and haemorrhage?

The microscopical appearance was particularly variegated in specimens taken from brown areas (macroscopically +) of lungs from the 16 rabbits with such changes. Four types of microscopical abnormalities were seen in 16 animals a) the bronchi filled with viscid mucus, leucocytes, and, occasionally foreign matter areas of purulent bronchitis and, in some cases, peribronchitis pneumonic foci b) atelectasis and emphysema c) focal haemorrhage and/or blood diffusely in the alveoli in addition there was d) alveolar oedema of varying extent. All the components were in some cases seen mixed together in others one of them predominated. Pneumonic areas were seen in 8 and alveolar oedema graded as + in 3 of these 16 rabbits.

This series comprised 57 animals. Alveolar oedema was seen in 19 out of 45 examined cases 12 animals with normal lung weights, no frothy fluid, and gross evidence of pulmonary oede-

ma were not examined microscopically (one of them, however was found to have a slightly increased water content and slight (+) radiographic pulmonary changes) In 17 of 19 cases with alveolar oedema the number of other positive criteria required in this series was sufficient to establish the diagnosis of pulmonary oedema. Exceptions were 2 animals without pulmonary oedema by the criteria used here which had alveolar oedema one of them had a water content of 84.8 % and white spots locally on the lung surface, (focal oedema?) The other one had hyperaemia and a spotty lung surface (++) macroscopically) unfortunately radiography was not performed but as the lung weight and water content were normal, only insignificant pulmonary oedema can have been present. Six animals with pulmonary oedema evidenced by all the other criteria used in this series (except for frothy fluid in 1 case) did not have alveolar oedema, all being overhydrated. The absence of alveolar oedema in overhydrated rabbits with high water contents and probable lung oedema was noted earlier in the dextran and adrenaline series. The difficulty of visualizing alveolar oedema in such cases has been described by Visacher et al. (246)

Pneumonia or bronchitis was seen microscopically in 11 of 28 rabbits with anuria for 48 hours and in 6 of 29 with anuria for a few hours. Seven out of 28 overhydrated rabbits and 10 out of 29 non-overhydrated rabbits had such infection. Seven out of 17 rabbits with pneumonia and bronchitis were

also judged as having pulmonary oedema. Bronchiolitis was not noted in any of the cases and periarterial oedema was seen in only 1 case.

Thus the microscopical picture was in this series of great value, particularly in the differential diagnosis. The disadvantage of the method was the difficulty of visualizing protein poor alveolar oedema and to exclude or establish the presence of pulmonary oedema in cases of co-existent alveolar oedema and pneumonia atelectasis, haemorrhage, or hyperaemia. In cases of such mixed pulmonary processes the diagnosis of pulmonary oedema was uncertain even when the alveolar oedema was relatively extensive and other criteria were desirable to support the diagnosis.

### *Pulmonary oedema and symptoms in vivo*

The appearance and behaviour of the animals gave no aid in determining the presence of pulmonary oedema pneumonia stagnation of secretion, or atelectasis but in animals that moved about relatively easily no very severe changes of the this kind were seen at autopsy.

In 34 rabbits which later on did not exhibit pulmonary oedema the respiratory rate averaged 52 (24—84) per minute and in 23 rabbits which later on exhibited pulmonary oedema according to the criteria used in this series, it averaged 50 (24—72) per minute. Bradypnoea persisted even in rabbits that died with all the other criteria indicating pulmonary oedema

Thus, the usual clinical symptoms of pulmonary oedema—tachypnoea and dyspnoea—could not be used as criteria for the diagnosis in the vagotomy series.

E.C.G. and heart rate. Immediately after the vagotomy the heart rate in 23 rabbits which later on exhibited pulmonary oedema by the criteria used here averaged 267 (187—340) beats per minute and in 34 rabbits without subsequently established pulmonary oedema it averaged 260 (180—308) beats per minute. At the bloodletting the average heart rate was in 5 rabbits with sufficient criteria of pulmonary oedema 248 (214—294) and in 26 rabbits without sufficient criteria of pulmonary oedema 266 (182—321) beats per minute.

Six out of 21 animals with E.C.G. disturbances eventually developed pulmonary oedema according to the criteria used here. Six of 19 rabbits in which pulmonary oedema was later on established by the criteria used here, had severe E.C.G. changes immediately after the vagotomy.

Thus, the heart rate and E.C.G. gave no guidance to the diagnosis of pulmonary oedema or the prognostication of its development.

The heart shadow was enlarged in 6 rabbits with pulmonary oedema and of normal size in 1 rabbit.

### *Criteria of pulmonary oedema*

The diagnosis of pulmonary oedema was difficult to establish in cases of pulmonary changes characterized by concurrent pneumonia atelectasis,

haemorrhage, hyperaemia, and oedema. In this series it was particularly difficult to distinguish rabbits with pulmonary oedema from rabbits with out pulmonary oedema. According to the rule that at least three criteria for the diagnosis of pulmonary oedema must be satisfied 30 out of 57 rabbits had pulmonary oedema: 4 of 15 slightly uraemic non-overhydrated, 7 of 14 severely uraemic non-overhydrated, 9 of 14 slightly uraemic overhydrated, and 10 of 14 severely uraemic overhydrated rabbits.

However at more critical examination this method for establishing the diagnosis of pulmonary oedema proved unsatisfactory in this series. It was found that 7 rabbits (nos. 3, 4, 20, 22, 52, 54 and 56) certainly had three positive criteria of pulmonary oedema (increased lung weight, macroscopical and microscopical pictures that could be compatible with pulmonary oedema, and/or in 2 cases, frothy fluid in the air ways) but proved to be cases of outspoken pneumonia. Microscopical examination showed pneumonic areas but no alveolar oedema: the changes were predominantly localized in the upper lobes or unilaterally and the water content was low. One of these rabbits had even four positive criteria of pulmonary oedema, but the water content was normal and there was no alveolar oedema.

With the requirement that at least five criteria for the diagnosis of pulmonary oedema should be satisfied, 15 cases would have been diagnosed as pulmonary oedema: 2 slightly uraemic non-overhydrated, 3 severely uraemic

non-overhydrated, 7 slightly uraemic overhydrated, and 3 severely uraemic overhydrated. On the other hand 4 rabbits with four positive criteria but in which X ray was not performed, would not have been included under this diagnosis, nor would rabbits with slight pulmonary oedema.

Other methods of assessment were therefore tried. The microscopical picture and the water content seem, according to Poulsen (206) and Diagrams 14—17 to provide the best aid in the differentiation between pneumonia and pulmonary oedema. In this series three positive criteria were required for the diagnosis of pulmonary oedema but a water content exceeding the upper limit of normal range or alveolar oedema established microscopically had to be one of them. An account of the result obtained by this method will be given below under the next heading.

This method, too, is not wholly satisfactory. The microscopical picture and the water content are in some situations misleading and the fulfilment of both criteria cannot be required, because at a very high water content visible alveolar oedema can be absent. Moreover as will be seen from the account of symptoms *in vivo* and post mortem findings, all the criteria used in this vagotomy series are uncertain in some situations. For this reason attempts at making a diagnosis of pulmonary oedema on the basis of various combinations of these criteria can lead to false results. The diagnosis of pulmonary oedema based on the criteria used here was sometimes in doubt.

a) Pulmonary oedema According to the criteria used here, 23 rabbits had pulmonary oedema. The diagnosis was in 8 cases (35 %) supported by all the six criteria in 7 cases (30 %) by five, in 7 cases (30 %) by 4 and in 1 case (5 %) by three criteria Six rabbits were not examined by X ray 2 with five criteria, 3 with four and 1 with three criteria of pulmonary oedema all of them had such marked macroscopical changes that X ray evidence would in all probability have been obtained if the examination had been performed.

The diagnosis was most uncertain in 5 animals in which four out of six or three out of five criteria were positive (nos. 10 21 47 49 and 51) None of these had pneumonia microscopically but nos. 21 and 51 had moderate hyperaemia besides alveolar oedema no 10 had haemorrhagic alveolar oedema and in no 49 the picture was peculiar in that the lungs were pink there was no frothy fluid in the air ways, and the microscopical examination showed no abnormalities, but the lungs were swollen and did not collapse the lung weight was 7.37 g per kg body weight, the water content was increased (84.0 %) and the pulmonary changes were clearly visible in the radiograph (oedema?)

The diagnosis was also uncertain in the rabbits that seemed to have co-existent pulmonary oedema and pneumonia. A slight increase in the leucocyte count was noted in half the rabbits with pulmonary oedema but foci of particularly numerous cells were seen in only 5 cases nos. 2 16 17 32

and 37 among which 4 had six and 1 had five positive criteria of pulmonary oedema (radiographs were missing) In 1 case (no 37) the water content was increased, 83.7 % but the animal was overhydrated and the alveolar oedema was modest.

The significance of haemorrhages and hyperaemia is still more difficult to evaluate, since they occur to some extent in virtually all animals with pulmonary oedema

b) No pulmonary oedema In 34 cases the number of positive criteria was less than three, or both the water content and the microscopical picture were normal, and hence pulmonary oedema was not considered to be present 12 of these rabbits had no positive criteria 0 had one criterion, 9 had two, 6 had three, and 1 had four positive criteria. The last mentioned 7 animals had pneumonia (see the foregoing) X ray examination was not performed in 3 and microscopical examination was not carried out in 12 With positive results of the two examinations that were not performed 2 of these 15 cases would have been diagnosed as having pulmonary oedema according to the criteria established for this series.

One of the rabbits died shortly after the vagotomy the lung weight was 7.13 g per kg body weight and macroscopically the lung surface was spotted with blue-pink brown there was no frothy fluid, the water content was normal and microscopical examination showed marked hyperaemia but no alveolar oedema unfortunately the radiograph was a failure.

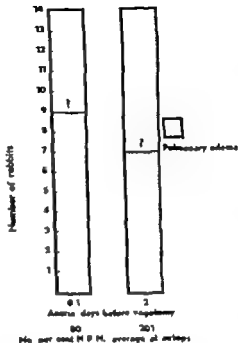
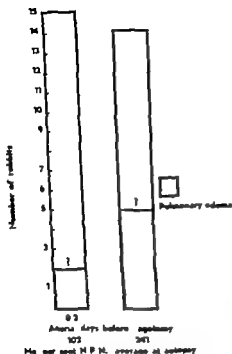


Diagram 12. Frequency of pulmonary edema after intra-ear infusion of Ringer solution, 18 % of the body weight for 1 1/2 hours, and bilateral agotomy in rabbits which had been anæmic for 0.1 and 2 days, respectively before the operation.

With the grouping of the rabbits by the criteria used in this series into those with and those without pulmonary oedema, borderline cases were of course inevitable but yet the division seems relatively satisfactory. The interpretation of the results was made difficult, partly by too early death from suffocation (8 out of 57 rabbits died from suffocation within 3 hours of the vagotomy without pulmonary oedema) and partly by the complicated picture that the pulmonary changes presented. In severe cases the diagnosis was easily established. The main difficulty was to exclude milder

degrees of lung oedema in the presence of other types of pulmonary changes.

This series shows the difficulties of using the same criteria for the diagnosis in all forms of pulmonary oedema. The experiences gained from this and the other series as regards the range of usefulness of the different methods of examination are discussed in chapter 10.

#### Pulmonary oedema and uræmia

The series comprised originally 61 animals, but the number was reduced to 57. Three non-overhydrated rabbits



with anuria for 48 hours and 1 overhydrated rabbit with anuria for a few hours were vagotomized but they died and as their dead bodies were not found until after rigor mortis had set in they were excluded from the series.

20 non-overhydrated rabbits (Table 24 and Diagram 12) Pulmonary oedema according to the definition used here, occurred in 2 of 15 animals which had been anuric for 0—4 hours at the vagotomy and in 5 out of 14 which had been anuric for 48 hours.

28 overhydrated rabbits (Table 25 and Diagram 13) Pulmonary oedema as defined here occurred in 9 out of 14 animals which had been anuric for 3—5 hours at the vagotomy and in 7 out of 14 which had been anuric for 48 hours.

Thus, in non-overhydrated animals the frequency of pulmonary oedema rose with increasing uraemia and in overhydrated animals it fell with in-

creasing uraemia. The difference between slightly uraemic and severely uraemic animals within the two series is not statistically significant however. The above figures for the frequency are uncertain as has been pointed out in the foregoing.

#### *Pulmonary oedema and overhydration*

The frequency of pulmonary oedema diagnosed by the criteria used here is higher for vagotomized rabbits with electrolyte fluid retention (52 %) than for rabbits without electrolyte fluid retention (24 %). In cases of slight uraemia (64 % and 13 % respectively) the difference could be almost significant ( $0.01 < p < 0.02$ ). The figures for frequency are uncertain however because of the difficulties in establishing the diagnosis of pulmonary oedema in this series.

*Survey* See page 152

## Detection and quantitative study of pulmonary oedema

In this study the main object was to induce slowly supervening pulmonary oedema, in an attempt to imitate clinical conditions. Pulmonary oedema developed mainly in association with four of the methods used, namely after hypervolaemia (blood, dextran) vaso-active drugs (adrenaline) pharmacological agents (barbiturates) and alterations of the peripheral nervous and respiratory systems (vagotomy). The object of using these methods was to try in various ways to throw light on the role of such factors together with uraemia and salt fluid retention in the causation of pulmonary oedema in rabbits with ligated ureters. The experiments were made at different times after the ureteral ligation, that is, at different degrees of uraemia. Half the animals in the adrenaline barbiturate, and vagotomy series were overhydrated with Ringer's solution. The experimental conditions were thus varied for the purpose of evaluating different criteria in the detection and quantitation of pulmonary oedema.

Suitable criteria for the diagnosis of experimentally produced pulmonary oedema have been discussed by Vischer et al. (246) and Poulsen (206). Vischer et al., after a short survey

concluded that, except for special purposes, gross observation and lung weight to body or heart weight ratios provide the most reliable practical methods for quantitation of lung oedema, whereas Poulsen recommends determination of lung weight and percentage of water in the lung. In the present experiments ten different methods (all mentioned earlier in the literature) of detecting pulmonary oedema were tried. Chest X-ray gross observation, observation and collection of frothy fluid in the air ways, measurements of lung weight, estimation of percentage water content in the lung, microscopical studies, observation of the appearance and behaviour of the animals, respiratory rate measurements, electrocardiography and determination of lung volume. The first six methods, in particular, were studied and compared with one another so as to ascertain the conditions under which the methods yield divergent results. A figure for the degree of correlation between the various methods has not been given, because this figure depends too much on the nature and degree, etc., of pulmonary oedema chosen (e.g. sudden or slow severe or slight generalized or

focal protein rich or protein poor rich in fluid or rich in blood uncom plicated or complicated with bleeding pneumonia hyperaemia atelectasis postmortem changes) Tables 10—25 will give some idea of the degree of correlation between the methods used in this study. The criteria of pulmo nary oedema found in each experi mental series was described in the re spective chapters a survey of the ob servations follows here.

### *Appearance and behaviour of the rabbits*

Acute sudden pulmonary oedema with violent dyspnoea and frothy fluid pouring forth from the mouth and nose, is a well known picture and easily diagnosed. It was only in a few ex periments, however that acute severe pulmonary oedema arose (after rapid injection of Narkotal or urea solu tion intravenously and occasionally after injection of adrenalline intramus cularly) in most experiments the de sired slowly supervening pulmonary oedema was obtained.

Slowly supervening pulmonary oede ma could be suspected from the ani mals appearance and behaviour in that they lifted their noses, breathing became distinctly audible, the depth and rate of respiration increased (see the next heading) the nostrils widen ed and the nose became moist. The animals sat still most of the time (par ticularly striking in the dextran series) except for sudden periods of restless ness (most distinct in the adrenalline series) notably premortally. Froth at

the nose appeared only as they were dying.

At slight degrees of pulmonary oede ma these signs could of course be lacking or insignificant. In animals which were ill or weak as a result of uraemia narcosis adrenalline injec tion or overhydration the presence of pulmonary oedema could be difficult to establish. Some of them would, for instance, lie down with irregular and feeble respiration whether pulmonary oedema was present or not.

The differential diagnosis was diffi cult between dyspnoea and other forms of pulmonary insufficiency resulting from for instance extensive atelecta sis, profuse pleural effusion or pneu monia. Moreover dyspnoea occurred at times in rabbits which had for in stance, stagnation of secretion or la ryngal paralysis (the vagotomy se ries) in animals during and after in fusion and in agitated animals (no tably in the adrenalline series but even in some normal rabbits). Overhydrated animals had profuse flow of fluid from their noses and in some cases it resembled frothy fluid. Rattling respi ration was heard mainly in vagoto mized animals indicating stagnation of secretion and air way obstruction similar but milder symptoms were present in overhydrated animals with profuse secretion in the air ways. Bloody flow from the nose was occa sionally seen in uraemic animals in the absence of pulmonary oedema. In the adrenalline series there was no corre lation between convulsions and the presence of pulmonary oedema.

The observations on the animals

appearance and behaviour were valuable, particularly so in sudden pulmonary oedema, but the assessment became increasingly hazardous the milder the pulmonary oedema or the weaker the animals' power of reaction, and the greater the number of differential-diagnostic factors that intervened. On the other hand, pulmonary oedema could in many cases be excluded by observing the animals, as experimental rabbits which looked and behaved like normal rabbits rarely had pulmonary oedema.

#### *Respiratory rate*

The relevant literature contains no studies on the respiratory rate in uraemic rabbits with pulmonary oedema. For rabbits with pulmonary oedema the average respiratory rate was higher than for those without pulmonary oedema in the 'Narkotal' and the adrenaline series but not in the vagotomy series. In the individual case, however, the respiratory rate was an unreliable aid to diagnosis, since many animals with pulmonary oedema had a normal or low respiratory rate and since animals with pulmonary processes other than oedema also had a high respiratory rate. The respiratory rate of normal animals in this study averaged 122 (72-220) per minute. Yet, some restless normal rabbits had a respiratory rate of up to 430 per minute which was higher than the range for rabbits with pulmonary oedema. The highest respiratory rate for those rabbits, 228 (92-380) was noted in the adrenaline series. For rabbits with slight pulmonary oedema in the dex-

tran series the respiratory rate averaged 128 (100-160) per minute.

The respiratory rate fell when the animals were at rest or placed on their backs. It fell with increasing uraemia and after 72 hours of anuria it averaged 79 (33-140) per minute. A respiratory rate lower than normal was noted after infusions, after vagotomy in association with narcosis, and pre-mortally. Highly uraemic rabbits had as a rule, if they developed pulmonary oedema, a high respiratory rate which added to the diagnostic value of the respiratory rate in cases of severe uraemia.

The respiratory rate can be a valuable aid to the diagnosis of pulmonary oedema, when the condition is not complicated by other pulmonary processes and the animals are undisturbed, moribund, anaesthetized, or vagotomized. A normal respiratory rate can be a useful aid in excluding pulmonary oedema.

#### *Electrocardiography and heart rate*

Severe electrocardiographic disturbances were very common in these experiments and were due partly to high degrees of uraemia (Chapter 5) and partly to other experimental conditions ('Narkotal' narcosis, adrenaline injections, vagotomy infusions). As an aid to the diagnosis of pulmonary oedema E.C.G. was found to be of very little value, as most of the rabbits with E.C.G. abnormalities did not have pulmonary oedema, and as many rabbits with pulmonary oedema had only slight E.C.G. changes.

The heart rate proved to be of no

certain diagnostic value, as it changed very little in association with pulmonary oedema and uraemia

The degree of an E.C.G. disturbance, on the other hand was found to be very useful in estimating the remaining survival time of a uraemic rabbit.

### *Chest X ray*

The only critical evaluation of chest X ray in experimentally produced pulmonary oedema has been made by Visscher et al (246) who in quoting Westermarck (252) state that the central density ordinarily interpreted as evidence for pulmonary oedema is almost certainly to be ascribed to a large extent to distended blood vessels that X ray evidence in some situation occurs only when the oedema has reached an advanced stage and that X ray density studies during life is subject to great uncertainty. The present author does not share this view on the ground of his own investigations. It will be seen from Tables 10—25 and Diagram 18 that in most cases the X ray evidence is well consistent with the other criteria, and that chest X ray is one of the most sensitive methods for evaluation of the oedema state.

However some difficulties attached to the method. In cases of very small and of very extensive X ray density changes, in particular it was difficult to make the differential diagnosis between oedema and pneumonia (the vagotomy series) pulmonary haemorrhage (the dextran and Narkotal series) and atelectasis (the vagotomy and the adrenaline series and overhydrated

animals). In the series of blood, dextran, and other infusions slight X ray changes, later unexplained at autopsy can be suspected to have been ascribable to hyperaemia. On the other hand, profuse pleural effusion without atelectasis gave no X ray changes as has been shown by Alwall & Lunderquist (to be published). The radiographic possibilities of distinguishing between pulmonary oedema on the one hand and pneumonia pulmonary haemorrhage, atelectasis, and hyperaemia on the other have not been brought up for consideration in this work.

The X ray method is uncertain in cases in which the oedema is localized mainly at the base of the lung or occurs in the form of numerous circumscribed lesions (the Narkotal and the dextran series). X ray was normal in 3 cases of acute sudden pulmonary oedema resulting from rapid injection of Narkotal solution intravenously or adrenaline solution intramuscularly despite the fact that frothy fluid poured forth from the nose and mouth during the X ray examination.

The number of films that were unsuccessful because of oblique centering or technical mishaps was small. Severely ill animals were affected even by very short examination in the hanging position. Many of the vagotomized animals could not breathe at all in this position. The lung surface visible in the radiograph diminished as the animals general condition deteriorated, when there were profuse amounts of ascitic fluid which raised the diaphragm and when the heart was greatly enlarged all this made the na-

assessment of pulmonary changes difficult in many cases. In uraemic overhydrated rabbits a density in the medial part between the heart and the diaphragm was occasionally seen, but no abnormalities were found at postmortem examination (Landerquist, to be published).

In animals that appeared to die spontaneously it was not practically possible to take radiographs, as desired, immediately before death. To be able to include a greater number of the spontaneously dead rabbits, films were therefore also taken of the animals in the agonal state or within five minutes of their last breaths. In uncertain cases X ray examination was not made.

At a check up of 18 animals killed by injection of air, bloodletting, or injection of "Narkotal" no X ray density changes in the lungs were seen within five minutes of death. In 2 rabbits, killed by injection of air and an anaesthesia respectively no pulmonary changes were seen within two hours but slight changes were noted within four hours of death.

Chest X ray is useful in experiments *in vivo*, when the differential diagnosis presents no difficulties.

#### Gross observation

According to Vlacher et al (210) gross observation and lung weight measurement provide the most reliable methods for quantitation of pulmonary oedema. Farber (83) used ocular inspection to study the development of pulmonary oedem in vagotomized animals initially petechial haemor-

rhages occurred, thereafter red discoloration, and at autopsy consolidated areas were seen. Poulsen (206) described the lungs in excessive oedema as full with more or less extensive hyperaemia. Less pronounced pulmonary oedema is not directly visible but must often be suspected in the presence of fairly extensive hyperaemia which usually precedes or accompanies oedema formation.

A comparison between macroscopical evaluation of pulmonary oedema, as graded and defined in this study (Chapter 4) and other criteria of pulmonary oedema of the kind used here has not been found in the relevant literature.

It will be seen from Diagram 18 and Tables 10—25 that gross observation was the most sensitive of the methods used here for diagnosis of pulmonary oedema. In all cases, except 1 there was gross evidence of pulmonary oedema when at least three criteria were fulfilled. It was the best method for detecting minute changes which could be suspected to evidence pulmonary oedema, notably those circumscribed local lesions that were seen most frequently in the "Narkotal" series.

It will also be seen from Diagram 20 and Tables 10—25 that with the macroscopical evaluation of pulmonary oedema as defined here, pulmonary oedema was diagnosed in excess. In many cases the gross evidence of slight pulmonary oedema was not supported by other criteria. This failure was due to difficulties associated with gradation and the differential diagnosis.

Gradation was difficult, quantita-

tively as well as qualitatively in the borderline area between pulmonary oedema and no pulmonary oedema a) the oedema might involve only a small part of the lung mostly the hilar or basal regions b) slight generalized changes of a type that are seen in a more marked form in cases of considerable oedema led to subjective assessment, c) other pulmonary processes might co-exist and hence the degree of pulmonary oedema could only be suspected (notably in the vagotomy series)

The differential diagnosis presented problems with respect to atelectasis pneumonia haemorrhages and/or hyperaemia. In some cases it was difficult to determine whether pulmonary oedema was present and still more difficult, as mentioned above, to estimate the extent of the oedema macroscopically if several processes co-existed. These difficulties were particularly noticeable in the vagotomized animals in animals with hyperaemia or numerous small haemorrhages (the dextran and the adrenaline series) and in the spontaneously dead rabbits with suspected postmortem changes in the lungs.

The macroscopical picture of pulmonary oedema was not similar in all the series. Small differences occurred, owing to the co-existence of several pulmonary processes, such as haemorrhages in the dextran series, hyperaemia in the adrenaline series focal changes in the Narkotal series and brown consolidated upper lobe changes with pneumonia etc., in the vagotomy series. This may necessitate a

special method of gradation for different forms of pulmonary oedema.

Gross observation is useful as a quick method which is expedient in examining all forms of pulmonary changes. Its inadequacy relates to the differential diagnosis and the subjective gradation.

### *Frothy fluid in the air ways*

Lulsada & Sarnoff (170) submitted dogs to tracheotomy and, in their lung oedema studies, noted spontaneous outpouring of frothy sputum in vivo. They found that this observation was deceptive in some experiments moderate oedema gave rise to early outpouring of frothy material in others, severe oedema did not. They decided that only data based on postmortem findings were to be relied upon in determining the presence and degree of pulmonary oedema. In grading the presence of frothy fluid they also employed sectioning of the parenchyma and squeezing of the lung. The gradation agreed well with the lung weight.

The present author noted enormous amounts of frothy fluid in association with acute pulmonary oedema after injection of adrenaline intramuscularly or after rapid intravenous injection of urea in animals whose general condition was good. In some cases, animals in a poor general condition became for instance severe uraemia died suddenly from severe pulmonary oedema evidenced by all the other criteria with only insignificant amounts of frothy fluid.

As will be seen from Diagram 18 and

Tables 10—25 the presence of frothy fluid in the air ways is in most cases well correlated with the other criteria of pulmonary oedema and frothy fluid occurred preferentially in animals with distinct evidence of pulmonary oedema by the other criteria. The presence of froth was less common than the other five criteria of pulmonary oedema used here.

Difficulties attached to the differentiation against aspirated mucus (the vagotomy series) and haemorrhages and secretion in the trachea and bronchi (uraemic and overhydrated animals with profuse tracheal secretion) Luisada & Sarnoff (170) found similar difficulties in vivo in overhydrated dogs. If frothy fluid alone was present in the bronchi and in small amounts, they found it difficult to distinguish between frothy fluid due to pulmonary oedema and frothy fluid that can occur on squeezing of normal lungs at autopsy. Frothy fluid in the air ways may possibly form post mortem (Chapter 8). Among 52 animals examined 21 hours after death, 19 % had frothy fluid in the air ways, while no frothy fluid was found in 49 animals which were examined before rigor mortis had set in.

In the Narkotal series it was found that severe uraemic animals with florid alveolar oedema at microscopic examination, had no or little frothy fluid in the air ways. Accordingly the composition of the exudate may possibly play a part in the formation of frothy fluid.

Observation of frothy fluid in the air ways, being a simple and quick

method, is a valuable aid to the diagnosis of marked pulmonary oedema.

### *Lung-weight*

According to Vischer et al (240) the lung weight, together with gross observation, is the most reliable method for diagnosis and quantitation of lung oedema. The values found in the present study agree relatively well with those reported by others (Chapter 4). Tables 10—5 show that the lung weight is generally well correlated with other criteria of pulmonary oedema. However the usefulness of the method has some limitations.

The lung weight per kg body weight changes noticeably with increasing body weight (Diagram 3). Poulsen (206) has shown that this is also the case in mice. It will be seen from the diagram that the experimental animals ought to be of approximately the same weight at the beginning of the experiment. To draw the line between animals without and animals with pulmonary oedema on the basis of the lung weight is difficult as will be seen from Courtice's (48) diagram. In these experiments, focal pulmonary oedema (occurred mainly in the Narkotal series) caused only slight rises in the lung weight. In the present study the lung weights of normal rabbits were taken as normal values. It may possibly have been preferable to use the lung weights of uraemic animals (Tables 7 and 8) as a material for comparison with bled uraemic animals, and the lung weights of animals after infusion of Ringer's solution (Tables 7, 12 and 13) as a material for com-



tively as well as qualitatively in the borderline area between pulmonary oedema and no pulmonary oedema a) the oedema might involve only a small part of the lung mostly the hilar or basal regions, b) slight generalized changes of a type that are seen in a more marked form in cases of considerable oedema led to subjective assessment, c) other pulmonary processes might co-exist and hence the degree of pulmonary oedema could only be suspected (notably in the vagotomy series)

The differential diagnosis presented problems with respect to atelectasis, pneumonia, haemorrhages and/or hyperaemia. In some cases it was difficult to determine whether pulmonary oedema was present and still more difficult, as mentioned above, to estimate the extent of the oedema macroscopically if several processes co-existed. These difficulties were particularly noticeable in the vagotomized animals, in animals with hyperaemia or numerous small haemorrhages (the dextran and the adrenaline series) and in the spontaneously dead rabbits with suspected postmortem changes in the lungs.

The macroscopical picture of pulmonary oedema was not similar in all the series. Small differences occurred owing to the co-existence of several pulmonary processes, such as haemorrhages in the dextran series, hyperaemia in the adrenaline series, focal changes in the Narkotal series and brown consolidated upper lobe changes with pneumonia etc., in the vagotomy series. This may necessitate a

special method of gradation for different forms of pulmonary oedema.

Gross observation is useful as a quick method which is expedient in examining all forms of pulmonary changes. Its inadequacy relates to the differential diagnosis and the subjective gradation.

### *Frothy fluid in the air ways*

Lusada & Sarnoff (170) submitted dogs to tracheotomy and, in their lung oedema studies, noted spontaneous outpouring of frothy sputum *in vivo*. They found that this observation was deceptive in some experiments moderate oedema gave rise to early outpouring of frothy material in others, severe oedema did not. They decided that only data based on postmortem findings were to be relied upon in determining the presence and degree of pulmonary oedema. In grading the presence of frothy fluid they also employed sectioning of the parenchyma and squeezing of the lung. The gradation agreed well with the lung weight.

The present author noted enormous amounts of frothy fluid in association with acute pulmonary oedema after injection of adrenaline intramuscularly or after rapid intravenous injection of urea in animals whose general condition was good. In some cases animals in a poor general condition because of for instance severe uraemia died suddenly from severe pulmonary oedema evidenced by all the other criteria, with only insignificant amounts of frothy fluid.

As will be seen from Diagram 18 and

Tables 10—13 the presence of frothy fluid in the air ways is in most cases well correlated with the other criteria of pulmonary oedema and frothy fluid occurred preferentially in animals with distinct evidence of pulmonary oedema by the other criteria. The presence of froth was less common than the other five criteria of pulmonary oedema used here.

Difficulties attached to the differentiation against aspirated mucus (the vagotomy series) and haemorrhages and secretion in the trachea and bronchi (uraemic and overhydrated animals with profuse tracheal secretion) Laisada & Sarnoff (170) found similar difficulties *in vivo* in overhydrated dogs. If frothy fluid alone was present in the bronchi and in small amounts, they found it difficult to distinguish between frothy fluid due to pulmonary oedema and frothy fluid that can occur on squeezing of normal lungs at autopsy. Frothy fluid in the air-ways may possibly form post mortem (Chapter 5). Among 52 animals examined 24 hours after death, 19 % had frothy fluid in the air ways, while no frothy fluid was found in 49 animals which were examined before rigor mortis had set in.

In the 'Narkotal' series it was found that severe uraemic animals with fibrous alveolar oedema at microscopical examination, had no or little frothy fluid in the air ways. Accordingly the composition of the exudate may possibly play a part in the formation of frothy fluid.

Observation of frothy fluid in the air ways, being a simple and quick

method, is a valuable aid to the diagnosis of marked pulmonary oedema.

### *Lung-weight*

According to Vlachet et al (1240) the lung weight together with gross observation, is the most reliable method for diagnosis and quantitation of lung oedema. The values found in the present study agree relatively well with those reported by others (Chapter 4). Tables 10—25 show that the lung weight is generally well correlated with other criteria of pulmonary oedema. However the usefulness of the method has some limitations.

The lung weight per kg body weight changes noticeably with increasing body weight (Diagram 3). Poulsen (206) has shown that this is also the case in mice. It will be seen from the diagram that the experimental animals ought to be of approximately the same weight at the beginning of the experiment. To draw the line between animals without and animals with pulmonary oedema on the basis of the lung weight is difficult, as will be seen from Courtice's (48) diagram. In these experiments, fatal pulmonary oedema (occurred mainly in the 'Narkotal' series) caused only slight rises in the lung weight. In the present study the lung weights of normal rabbits were taken as normal values. It may possibly have been preferable to use the lung weights of uraemic animals (Tables 7 and 8) as a material for comparison with bled uraemic animals, and the lung weights of animals after infusion of Ringer's solution (Tables 7, 14, and 18) as a material for com-

parison with overhydrated animals. Another difficulty attaching to the use of the lung weight in the detection of pulmonary oedema is that poorly bled animals (usually moribund) without pulmonary oedema fall into the same weight-class as well bled animals with slight pulmonary oedema.

The borderline between animals without and animals with pulmonary oedema is still more difficult to establish in spontaneously dead rabbits. Durlacher et al. (73) have shown that the lung weight increases gradually after death the rate of increase depending upon the manner of death. As a material of comparison for the lung weight in non bled animals were used 39 rabbits which died spontaneously in uraemia and were examined within 90 minutes after death. These "normal values" can of course be only approximate, as the animals died from different causes (overhydration, injection of Narkotal and adrenalline, vagotomy, uraemia) and were examined at different times (though usually immediately) after death. The variation in the time interval between death and examination could not be avoided. Continuous night supervision of the animals could not be arranged in these experiments and therefore the times of spontaneous deaths could sometimes only be established approximately but only animals whose bodies were warm and soft when found, have been included in the material (that is those that had been dead for about  $1\frac{1}{2}$  hours at the longest). Further investigation into postmortal changes would be valuable. This problem is

also of great importance with respect to autopsies in man. Bloodletting after death before any postmortal pulmonary oedema has developed, would provide a possibility to study the problem more closely.

Durlacher et al. found that the quantity of oedema present 3 hours after death varies with the method of sacrifice, the route of administration of the lethal agent, the temperature, and the intratracheal pressure. In the present study it was noted that the lung weight increased only slightly after death in some animals and markedly in others (Chapter 4). The rate of the postmortal increase of the lung weight is influenced by factors which are in sufficiently known. The position of the body after death seems to play a part as the lowermost parts of the lungs are always the heaviest postmortally. Marked hyperaemia in the lungs at the moment of death may conceivably increase the tendency to postmortal pulmonary oedema (Chapter 4). Since postmortal transport of blood or serum to the lungs is probably a prerequisite for postmortal pulmonary oedema (Chapter 5) postmortal clotting time and pressure in the blood vessels could be important factors. Overhydrated rigid animals were found to have the highest lung weights, which could indicate that overhydration is another important factor.

Poulsen (206) has suggested that a weight increase in the lungs may be due to other factors besides oedema fluid, e.g. congestion and inflammation. This is confirmed by the present

investigations in which animals with pneumonia (the vagotomy series and 3 rabbits with pneumonia whose ureters had not been ligated) pulmonary haemorrhages (notably the dextran series) and hyperaemia (the adrenal line and the vagotomy series, and some animals that died spontaneously from injection of air intravenously or 'Narkotal' injection) had lung weights exceeding the upper limit of normal range. In animals with white-spotted lungs, seen after overhydration, the lung weights were not increased.

In association with experimentally produced non-focal pulmonary oedema without hyperaemia, pulmonary haemorrhages, or postmortal changes of any noteworthy degree the lung weight is a good method for evaluation of pulmonary oedema. The longer the interval after death the less the possibility to diagnose or quantitate pulmonary oedema by using the lung weight in non bled animals but the presence of pulmonary oedema can be excluded if the lung weight after death is low.

#### *The water content of the lung*

Determination of the water content in the lung has been made by various techniques and opinion differs as regards the usefulness of the method (Chapter 4). The heat lamp technique does not seem to have been used for determination of the water content of the lung in experimental pulmonary oedema. This technique seems to work well for the drying of 1 mg tissue but is less expedient for the drying of whole blood (Chapter 4 Chapter and Table

7) It will be seen from Tables 10—25 that there was a good correlation between the water content and the amount of oedema fluid noted otherwise. The water content alters very little at increased body weight, as has been shown in mice by Poulsen (200). The method has some disadvantages, however.

The determination of the water content uses up one lung. When gross pulmonary changes involve predominantly one lung which happens in rare cases a choice must be made between water content and histological verification (Reichman [213]).

It will be seen from Tables 10—25 that several overhydrated rabbits had increased water contents in their lungs, up to 85 % without showing any other evidence of pulmonary oedema. In these cases, the fluid was possibly present in blood and lung tissue and did not occur in the alveoli. In highly overhydrated animals the water content will therefore be a less reliable criterion of pulmonary oedema.

Poulsen (200) showed that in mice the water content of the lung averaged 77.11 % of blood 78.5 % and of oedema fluid from the lung 94.9 %. He concluded that it seems evident that the water content of lungs showing a weight increase due exclusively to an increased accumulation of blood does not differ essentially from that of normal lungs. On the other hand, a weight increase caused by oedema fluid will raise considerably the water content of the lungs. Poulsen considered that the water content could therefore be a useful means of diagnosing

pulmonary oedema against hyperaemia and inflammation. The present investigation has confirmed the low water content of the lung in pneumonia (the vagotomy series and 3 animals with pneumonia whose ureters were not tied) haemorrhages into the lung parenchyma (the dextran series) and hyperaemia (the adrenaline and the vagotomy series, and some animals that died spontaneously after injection of Narkotal or air intravenously) as well as the high water content of the lung in cases of seemingly uncomplicated pulmonary oedema. Atelectatic lungs of animals with profuse pleural effusion following overhydration on the other hand were in many cases rich in fluid. White spotted lungs, which were seen in overhydrated animals, showed in many cases an increased water content (Diagram 16).

Thus, the water content is useful in the differential diagnosis between pulmonary oedema on one hand and hyperaemia pneumonia and haemorrhages into the lung parenchyma on the other. Pulmonary oedema cannot be safely excluded on the basis of a normal water content, for in pulmonary oedema with co-existing hyperaemia haemorrhage or pneumonia the water content can be normal. In several rabbits belonging to the dextran Narkotal and vagotomy series the water content was certainly normal but they had pulmonary oedema evidenced by four or five other criteria. The alveolar oedema fluid may be fibrinous, it may have a high protein content and hence a low water content, or it can be sparse in relation to blood

cells and inflammatory tissue. Consequently a low water content of lungs with high weights is a guide to diagnoses other than pulmonary oedema, and was thus used in the vagotomy series as a means of excluding other pulmonary processes. For further discussion, see Chapter 9.

In comparison with the postmortal increase in the lung weight, the rise in the water content post mortem was moderate (Chapter 5). In 71 % of 21 rigid, non overhydrated rabbits the water content was below the upper limit of normal range for spontaneously dead rabbits. 38 % had a lung weight below the upper limit of normal range. In no case did the water content post mortem exceed 86.3 % as it may do in rabbits with moderate pulmonary oedema. A high water content should therefore be more useful than a high lung weight as supporting evidence for the presence of pulmonary oedema in vivo in rigid animals. In some rigid animals overhydrated in vivo however the water content reached 88 % (Diagram 17) and this fact limits the usefulness of the water content in the diagnosis of pulmonary oedema in overhydrated rigid animals.

The method is particularly valuable in the differential diagnosis

*Estimation and measurement of the water content.* The water content of the lung was estimated roughly at autopsy by inspection of the lung surface, the section surface, and the amount of fluid obtained by squeezing and cutting of the lung. The estimated water content was graded as +, ++ and +++ Comparison was made between this gradation and the water content measured by drying of the lung. On

451 occasions the correlation was  $0.62 \pm 0.02$ . A water content higher than the estimated one was found chiefly in some overhydrated animal and also in some spotonized animals. A water content lower than that expected from the estimation was recorded for spontaneously dead animals and animals with hyperaemia, pulmonary haemorrhages, and bronchitis.

### *Microscopical studies*

According to Vlascher et al. (246) microscopical studies have proved disappointing in experimental lung oedema. They frequently sectioned lungs with acute massive oedema and found that there was no stainable material in the alveoli. Koenig (147) showed that in early acute lung oedema the oedema fluid may be nearly protein-free. According to Vlascher et al., the only early evidence of such lung oedema that is visible microscopically is interstitial wall thickening, because protein-free fluid in alveoli is undetectable in conventional histological preparations.

In this study as well, the microscopical picture was virtually normal (even without interstitial wall thickening) in animals which had marked pulmonary oedema by all the other criteria (Figs. 26 and 27). In animals with marked pulmonary oedema following infusions of large doses of dextran and in overhydrated rabbits with pulmonary oedema in the adrenalinic and the vagotomy series the absence of alveolar oedema was particularly striking. Although there was no precipitated protein in the alveoli, the shape of the alveoli, the thickness of the alveolar septa, and the presence of red cells and

alveolar phagocytes in the alveoli could in some cases give rise to the suspicion of pulmonary oedema. Since however these findings are not specific for pulmonary oedema, the presence of visible precipitated protein in the alveoli (alveolar oedema) was the only factor considered here. Some experiments failed in which an attempt was made at obtaining better visualization of protein poor alveolar fluid by using a different fixative (Carnoy's fluid).

Moreover alveolar oedema was seen around haemorrhagic areas and foci of infection, and when numerous small foci were present as in the vagotomy 'Narkotal' and dextran series, the differential diagnosis against alveolar oedema of the haemorrhagic lung oedema type was often difficult. In this situation it might have been expedient to give antibiotics to vagotomized animals as an attempt at overcoming the pneumonia. Antibiotics were given for this purpose by Alwall et al. for instance to 'Narkotal' treated animals. As regards the differential diagnosis, the greatest difficulties were presented by the vagotomy series, in which some animals had co-existing pulmonary oedema, pneumonia atelectasis, hyperaemia, and haemorrhages. In this series it was also very difficult to estimate microscopically the extent of pulmonary oedema. In spite of these shortcomings, the microscopical examination was in many cases of decisive importance in establishing the type of pulmonary changes.

The dextran, 'Narkotal', adrenalinic and vagotomy series included animals which had alveolar oedema but in

pulmonary oedema against hyperaemia and inflammation. The present investigation has confirmed the low water content of the lung in pneumonia (the vagotomy series and 3 animals with pneumonia whose ureters were not tied) haemorrhages into the lung parenchyma (the dextran series) and hyperaemia (the adrenaline and the vagotomy series, and some animals that died spontaneously after injection of Narkotal or air intravenously) as well as the high water content of the lung in cases of seemingly uncomplicated pulmonary oedema. Atelectatic lungs of animals with profuse pleural effusion following overhydration on the other hand were in many cases rich in fluid. White spotted lungs, which were seen in overhydrated animals showed in many cases an increased water content (Diagram 15).

Thus the water content is useful in the differential diagnosis between pulmonary oedema on one hand and hyperaemia pneumonia and haemorrhages into the lung parenchyma on the other. Pulmonary oedema cannot be safely excluded on the basis of a normal water content for in pulmonary oedema with co-existing hyperaemia haemorrhage, or pneumonia the water content can be normal. In several rabbits belonging to the dextran Narkotal and vagotomy series the water content was certainly normal but they had pulmonary oedema evidenced by four or five other criteria. The alveolar-oedema fluid may be fibrinous, it may have a high protein content and hence a low water content or it can be sparse in relation to blood

cells and inflammatory tissue. Consequently a low water content of lungs with high weights is a guide to diagnoses other than pulmonary oedema and was thus used in the vagotomy series as a means of excluding other pulmonary processes. For further discussion see Chapter II.

In comparison with the postmortal increase in the lung weight the rise in the water content post mortem was moderate (Chapter 5). In 71 % of 21 rigid, non-overhydrated rabbits the water content was below the upper limit of normal range for spontaneously dead rabbits. 38 % had a lung-weight below the upper limit of normal range. In no case did the water content post mortem exceed 86.5 % as it may do in rabbits with moderate pulmonary oedema. A high water content should therefore be more useful than a high lung weight as supporting evidence for the presence of pulmonary oedema in vivo in rigid animals. In some rigid animals overhydrated in vivo however the water content reached 88 % (Diagram 17) and this fact limits the usefulness of the water content in the diagnosis of pulmonary oedema in overhydrated rigid animals.

The method is particularly valuable in the differential diagnosis

*Estimation and measurement of the water content.* The water content of the lung was estimated roughly at autopsy by inspection of the lung surface, the section surface, and the amount of fluid obtained by squeezing and cutting of the lung. The estimated water content was graded as ++, +, and +++. Comparison was made between this gradation and the water content measured by drying of the lung. On

the chest was contracted (muscle cramp) and large when it was expanded.

### Discussion

The quantitation of the edema state is a difficult problem at any site. It is particularly so in the lung because the organ is relatively inaccessible in the intact animal and because standards of reference are difficult to establish (Vischer et al.) In my experience, the difficulty in establishing which animals have and which have not pulmonary oedema is due to three factors

a) There is no available method by which, under any conditions, pulmonary oedema can be confidently diagnosed (Table 26). So as not to be too dependent upon one method with its weak points, and to ensure better support for the diagnosis in doubtful cases, I have used six methods simultaneously for the diagnosis of pulmonary oedema.

b) The borderlines are vague. With the requirement that the diagnosis should be incontestable many cases of slight pulmonary oedema would be excluded and, again, if all mild cases as well were to be taken into account, some doubtful borderline cases would be included. The use of both methods with all transitions has been reported in the literature. In this investigation it was required that three out of six given criteria should be positive. The reasons why the number of positive criteria required was not fixed at four, five, or six were the desire to consider less extensive pulmonary oedema, as acute violent forms were rare in these series,

and the risk that if any one method of examination was incidentally missing or gave a false negative result, cases of frank pulmonary oedema would not have been included.

c) Many pulmonary processes can co-exist with pulmonary oedema. This difficulty is most readily overcome if the nature of these processes is known. A method for diagnosis can then be chosen which gives different results for pulmonary oedema and the respective concurrent process (e.g. the water content in association with pulmonary oedema and with pneumonia). If many pulmonary processes of varying extent co-exist, as in vagotomized animals, the difficulties can be great. Several criteria are then necessary including microscopical evidence, to enable a reasonably confident diagnosis.

To choose the best method for the detection of pulmonary oedema, with few mixed and few wrong diagnoses, is difficult. The reason for this is that the diagnosis of pulmonary oedema is difficult in the individual case, and the advantages and disadvantages of the methods do not have the same influence under different experimental conditions (Diagram 18). It will be seen from this diagram that different methods of examination give a different frequency of pulmonary oedema, that the correlation between them varies with different forms of pulmonary oedema, and that, with respect to the results, no one procedure truly agrees with the method of using three positive criteria for the diagnosis of pulmonary oedema.



which the diagnosis of pulmonary oedema was not supported by a sufficient number of other criteria. An explanation of this besides the one given above was that the changes occurred locally in one part of the lung and that specimens for microscopical examination were taken from this part.

Quantitative and qualitative evaluation of the alveolar oedema was made only from the macroscopically most severely changed part of the lung and the gradation will therefore mirror the maximal rather than the average amount of alveolar oedema. Even with the naked eye it could be seen on the slides that accumulation of oedema fluid was greater in the hilar region than peripherally towards the lung margins. Specimens from both the upper lobe and the basal parts in the same rabbit showed regularly more alveolar-oedema fluid basally.

The lungs of rabbits with no gross abnormalities no frothy fluid, and normal lung weight were as a rule not examined microscopically. Specimens from such lungs were taken in each series, however and a total of 75 seemingly normal lungs were examined. In 44 of these the microscopical picture was also wholly normal. Among the remaining 31 were seen areas of bronchiolitis in 9 small areas of atelectasis in 8 a few focal haemorrhages in 6 slight hyperaemia in 4 areas of bronchitis in 3 and interstitial perivascular oedema in 1. None of these rabbits exhibited alveolar oedema. If all the unexamined lungs (125 cases, Tables 10—25) had contained alveolar

oedema fluid, only 3 cases would have been diagnosed as pulmonary oedema by three criteria as required here.

In postmortal pulmonary oedema the alveolar oedema was protein rich, the alveoli rarely contained red blood cells and alveolar phagocytes, and even peripheral alveoli could be filled with oedema fluid (Chapter 5). In spontaneously dead warm and soft rabbits the presence of this protein poor oedema fluid in a great number of alveoli in the hilar or the basal region, containing red cells and alveolar phagocytes, may support the suspicion that pulmonary oedema was present *in vivo* and not only *post mortem*.

Microscopical examination is of value in the differential diagnosis and less useful in the diagnosis and gradation of pulmonary oedema.

#### *Lung-volume and specific gravity*

The method was used initially in this study but was abandoned for several reasons. The air disappeared relatively quickly even from an intact lung and very quickly at the least leakage, which easily occurred. In non-bled animals the lung weight increases after death, at the same time as the air content, and hence the volume, decreases, so that the specific gravity will be greatly dependent upon the length of time that elapses between death and autopsy. Atelectasis, pneumonia, and other pulmonary processes cannot be detected by this method. Increased lung volume can occur without pulmonary oedema, and pulmonary oedema can occur in a normal sized lung. A lung that has been immersed in water for a while would hardly provide suitable material for determination of the water content. The lung volume varied with the method of sacrifice, in that the lungs became small when

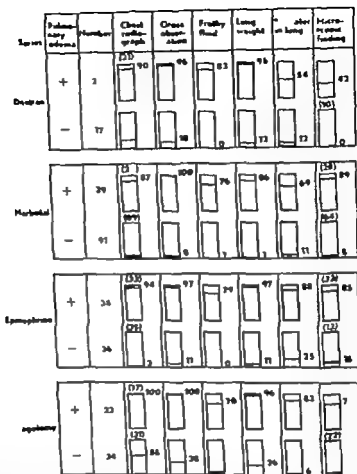


Diagram 18. Data from the dextran, "arkotal" epinephrine, and gotsky series. The number of rabbits with and without pulmonary edema, respectively. In the three first-named series, three criteria were sufficient to establish the diagnosis of pulmonary edema, whereas in the gotsky series the three criteria of pulmonary edema should include microscopical alveolar edema or water content above the upper limit of normal range. The occurrence of the following criteria is expressed as percentage (shaded black parts of the bars). Pulmonary changes which may represent edema (chest radiograph) macroscopical changes at autopsy which indicate pulmonary edema (gross observation); frothy fluid in the air way lung-weight in g per kg body weight above the upper limit of normal range; lung-weight; water content of the lung above the upper limit of normal range (% water in lung) and microscopical alveolar edema (microscopical findings). The occurrence of these criteria is also expressed numerically. The figures in parentheses denote the absolute number of animals in each group when it deviates from the numbers set out in the column headed "Number".

Table 28. Schematic survey of the sources of error in the diagnosis of pulmonary oedema in these experimental series

	Diagnosis				
	Chest X-ray	Gross observation	Prophy fluid if the air-way	Lung weight	Water content in the lung
Non-diagnosed pulmonary oedema	Focal		Small amount in the bronchi	Focal	Haemorrhagic?
	Basal				Hyperaemic?
	Very acute		Spontaneous death before formation of frothy fluid		Focal
			Viscid oedema fluid?		
Other pulmonary changes wrongly diagnosed as pulmonary oedema			In vivo		
	Atelectasis (pleural effusion)	Hyperaemia	Bronchitis	Pneumonia	Increase of extra alveolar fluid in the lungs
	Bleedings in the lungs	Numerous small haemorrhages	Stagnation of secretion	Hyperaemia	Numerous small haemorrhages or small areas of pneumonia
	Hyperaemia	Coexisting mixed lung processes, bleedings, hyperaemia, pneumonia and atelectasis	Bleedings in the air ways	Bleedings in the lungs	(Postmortal oedema)
	Pneumonia	(Postmortal oedema)		(Postmortal oedema)	

the other of pulmonary oedema with evidence of for instance large haemorrhage)

The following facts favour the three-criteria method a) The dependence upon one method with its shortcomings will be less (no one method would have been successful on all occasions in these experiments) b) Rabbits with two out of six criteria of pulmonary oedema seldom seemed, at subjective estimation, to be cases of pulmonary oedema after all c) Rabbits with hyperaemia or haemorrhages and three positive criteria of pulmonary oedema had such marked hyperaemia or such profuse haemorrhages into the lung parenchyma that the probable borderline was attained between hyperaemia and pulmonary oedema and between haemorrhages and haemorrhagic pulmonary oedema, respectively The method failed, however with respect to pneumonia (see the vagotomy series)

Another possibility that of combining the water content and the lung weight, has been suggested by Poulsen (206) In Diagrams 14—17 are listed 745 rabbits (from preliminary experiments as well) whose lungs were examined by both lung weight and water content To illustrate the influence of different pulmonary processes on the lung weight and water content the essential pulmonary change (diagnosed microscopically and/or macroscopically) in each rabbit has been marked out as follows A dot under the letter indicates that the rabbit had three positive criteria of pulmonary oedema but that some other

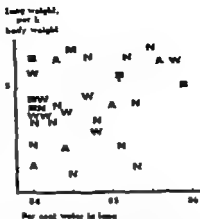


Diagram 15. The predominating types of pulmonary changes observed by macroscopical and/or microscopical examination of lung weight below 5.50 per kg body-weight and a water content above 84% A = atelectasis, B = bleedings, M = postmortal changes, N = normal, W = white-spotted lung surface. Of these, "W" refers to gross examination alone The presence of at least three criteria of pulmonary oedema is indicated by dot under the respective letter

pulmonary change predominated It will be seen from Diagram 15 that high water contents and normal lung weights can occur in rabbits whose lungs are normal macro- and microscopically and in those that have the type of pulmonary changes characterized by atelectasis haemorrhages, and white-spotted lung surface. There is only 1 case of suspected pulmonary oedema in this group Diagram 16 shows the dominating pulmonary change at high lung weight but normal water content. This group includes cases of pneumonia haemorrhages, hyperaemia and postmortal changes but few cases of outspoken pulmonary oedema. There are, how

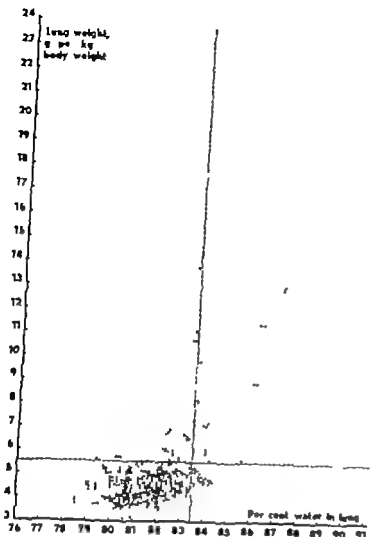


Diagram 14 Relation between lung weight in g per kg body weight and the water content of the lung as a percentage in 748 rabbits from all the experimental series

With the method of taking three criteria out of six as evidence for pulmonary oedema, the following differential-diagnostic difficulties arose. *Pneumonia* (the vagotomy series) can cause an increased lung weight frothy fluid in the air ways as well as radiological macroscopical and microscopical changes which may also occur in pulmonary oedema. *Haemorrhage* into the lung parenchyma (the dextran series) may increase the lung weight and give rise to macroscopical and radiological changes as in pulmonary

oedema. *Hyperaemia* can cause increased lung weight slight macroscopical and presumably slight radiological changes. So the problem of differentiating completely between rabbits with and rabbits without pulmonary oedema is not solved with the technique of using three positive criteria. Moreover two rabbits with pulmonary oedema evidenced by three though different criteria may exhibit a completely different microscopical picture (in one it may be that of slight uncomplicated pulmonary oedema in

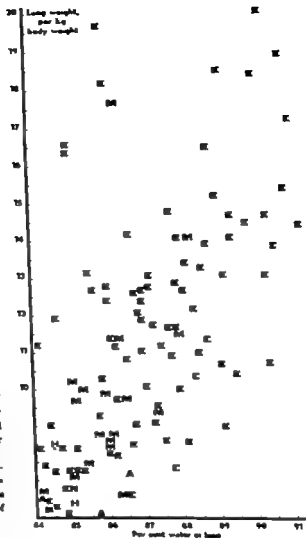


Diagram 17 Predominating types of pulmonary changes observed by macroscopical and/or microscopical examination: 1 lung-weight over 65 g per kg body-weight and water content above 65% B=bleeding, C=edema, H=hyperemia, M=pericardial changes, V=normal. The presence of at least three criteria of pulmonary edema is indicated by dot under the letter H.

have not been included in Diagram 16 and 17 respectively.

Diagrams 15—17 support the view that a combination of lung weight and water content of the lungs forms a good basis for evaluation of pulmonary oedema but that in marked hy-

peremia, pulmonary haemorrhages, and/or pneumonia further criteria, notably microscopical evidence, may be necessary as a means of establishing the presence of pulmonary oedema and evaluating the oedema state with reasonable probability.

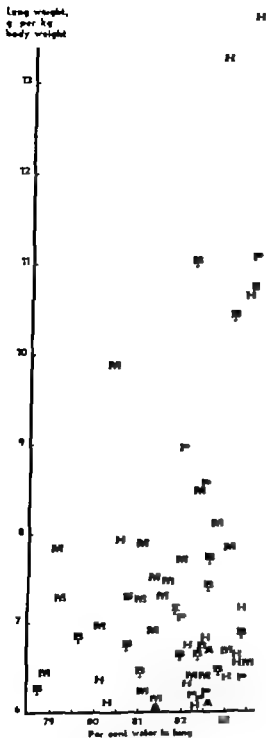


Diagram 16. Predominating pulmonary changes observed by macroscopical and/or microscopical examination at a lung weight over 6 g per kg body weight and a water content below 83.5 %. B=bleeding, E=edema, H=hyperaemia, M=postmortal changes, P=pneumonia.

ever a great number of rabbits with pneumonia hyperaemia, or haemorrhages which had three to six positive criteria of pulmonary oedema (indicated by a dot under the letter). Accordingly the water content can be normal in spite of macroscopical and radiological changes as in pulmonary oedema frothy fluid in the air ways, alveolar oedema microscopically and increased lung weight. The question as to in which of these cases pulmonary oedema actually co-existed with pneumonia hyperaemia or haemorrhages is difficult to answer conclusively. Diagram 17 shows the predominant pulmonary change in rabbits with high lung weights and high water contents. Pulmonary oedema by gross and microscopical evidence was the predominant change even though hyperaemia and haemorrhages were features of the picture in most of these cases. Postmortal pulmonary oedema was mostly found in association with a low water content but the highest water contents were noted in 5 severely overhydrated (25 % of the body weight) rabbits which after a few days of uraemia died in uraemia and were examined about 12 hours after death. For want of space, 1 rabbit with pneumonia a lung weight of 18 g per kg body weight, and a water content of 81 % and 2 rabbits with pulmonary oedema and lung weights of 23 g and 24 g per kg (Diagram 14)

pneumonia. The presence of at least three criteria of pulmonary oedema is indicated by dot under the letters B, E, H and P

## The uremia factor and the fluid factor

## The uraemia factor

Under these experimental conditions, uremia per se seems not to be sufficient to cause pulmonary oedema other factors must be present.

The question whether increasing uraemia would increase the susceptibility to pulmonary oedema, was studied by experiments on rabbits in which, after bilateral ureteral ligation, lung oedema was produced by four methods known to be expedient for this purpose. It was found that increasing uraemia increased the tendency towards lung oedema after infusions of dextran solution and after Narkotal anaesthesia whether the animals were overhydrated or not the above mentioned effect of uraemia was insignificant in non-overhydrated vagotomized rabbits and absent in the experimental series of overhydrated vagotomized rabbits, as well as after adrenaline injection, whether the animals were overhydrated or not.

The results seem to give rise to the following speculations

a) Perhaps high-degree uremia increases the susceptibility to pulmonary oedema under certain conditions only such as an enlarged plasma vol-

ume (the dextran series) · membrane changes (the Narkotal series) or air way obstructions, etc. (the vagotomy series)

b) The term uraemia is used here as a synonym of the disorders that arise after ureteral ligation, for instance an elevated  $\text{P}_{\text{CO}_2}$  level, disturbances of the acid base and the electrolyte balance the uraemia damages mucous membranes and the myocardium and causes respiratory and circulatory disturbances. It is not possible to single out any one of these factors as being especially capable of predisposing the animals to pulmonary oedema. On the other hand, ascites occurs after ureteral ligation the tendency towards formation of ascites results in displacement of fluid to the peritoneal cavity at overhydration, whereby the risk of pulmonary oedema may be reduced.

c) Unspecific factors may influence the frequency of pulmonary oedema under otherwise similar conditions. The fact that highly uraemic animals were more markedly affected by the Narkotal narcosis than slightly uraemic ones may possibly have contributed to the higher frequency of pulmonary oedema in the first mentioned



### *Range of usefulness of different methods for examination*

From the experience gained in these experiments the methods of examination applied here seem to have the following range of usefulness

- 1 The method for the diagnosis of pulmonary oedema must be chosen with a view to the purpose of the investigation and the method used in producing pulmonary oedema.
- 2 At *in vivo* investigations, X ray examination should be used and checking made by "clinical" assessment
- 3 In acute violent pulmonary oedema evaluation of frothy fluid in the air ways seems to suffice
- 4 When the recording of insignificant pulmonary changes is also desirable and/or great exactness is not required simple assessment of the gross appearance of the lungs and the presence of frothy fluid is preferable
- 5 If during the experiments, pulmonary oedema and pneumonia or haemorrhages in the lungs should occur only to a small extent and the animals can be bled lung weight measurement is the superior method for evaluation of pulmonary oedema
- 6 If the experimental conditions are such that not only pulmonary oedema but also hyperaemia pulmonary haemorrhage, and/or pneumonia may be thought to occur determination of the water content of the lungs is the best method in combination with lung weight measurement and, if desired, microscopical examination.
- 7 If several different pulmonary processes in various combinations should occur as in vagotomized animals microscopical examination is necessary
- 8 In rabbits with rigor mortis, pulmonary oedema developed *in vivo* can be suspected when there are profuse frothy fluid in the air ways, distinctly increased water content in the lungs, and thin alveolar-oedema fluid with alveolar phagocytes and red cells in the alveoli and when there are bilateral changes consistent with pulmonary oedema localized mainly in the hilar region.
- 9 Pulmonary oedema in normal or slightly ill rabbits can most readily be excluded by clinical assessment
- 10 In increasing uraemia a slight rise in the frequency of haemorrhages, atelectasis, and pneumonia was noted in some series. Therefore the need of several methods including microscopical examination and estimation of water content increased with increasing uraemia in these series.
- 11 Only by using several methods simultaneously can a fairly exact qualitative and quantitative evaluation of pulmonary oedema be made in rabbits

came very poor after amounts equaling only 15 % of their body weights. This can be due to the effect of such factors as haemolysis, cerebral oedema, intoxication, hyperglycaemia or hyponatraemia. Pulmonary oedema was not noted.

The experimental conditions limit to some extent the possibilities to draw a conclusion about uraemia as a predisposing factor in pulmonary oedema. In some experiments the overhydration was carried out such a long time after the ureteral ligation that the remaining length of life might have

been too short for pulmonary oedema to develop.

The conclusion that can be drawn from these experiments is, mainly that overhydration in association with uraemia in animals with both their ureters ligated produced pulmonary oedema only if the overhydration meant an increase of the blood volume. Other factors than uraemia must be present. Salt and fluid retention increased the susceptibility to pulmonary oedema after Narkotal narcosis, adrenaline injection, and vagotomy in uraemic rabbits.

group. The stress of the adrenaline effect in already severely ill animals may shorten their lives so that there will be no time for pulmonary oedema to develop before death occurs. The same may be the case after vagotomy, particularly in those groups in which the operation was made a relatively long time after the ureteral ligation that is, in cases of severe uraemia and/or serious infectious complications which possibly could have been prevented by antibiotic treatment.

These considerations lead to the conclusion that uraemia following ligation of the ureters can be a factor which under some conditions increases the frequency of pulmonary oedema and under other conditions lessens it.

### The fluid factor

After vagotomy, Narkotal narcosis and adrenaline injection animals with salt and fluid retention developed pulmonary oedema more readily than did animals without salt and fluid retention irrespective of the degree of uraemia. This confirms other authors' observations that salt and fluid retention increases the susceptibility to pulmonary oedema under various experimental conditions. In the 3 experimental series mentioned above the degree of overhydration was relatively low; the amount of administered fluid was 15 % of the animals' body weight (less than 1.7 ml per kg body weight were infused per minute).

Despite administration of blood in amounts that were twice those of the animals' circulating blood, slight pul-

monary oedema developed in only a few cases. On the other hand, when the plasma volume was increased by the infusion of dextran the frequency of pulmonary oedema produced was higher than after administration of the same amount of blood. This may be interpreted as showing that an increase of the plasma volume is the essential factor in the development of pulmonary oedema at overhydration. The result may however depend upon other effects of the added dextran, such as anaemia due to dilution, altered capillary permeability and/or altered composition of the extracellular fluid.

The extracellular fluid was increased by infusion of mannitol solution or Ringer's solution. Mannitol infusion led to hyponatraemia and had a toxic effect at dose levels of 15 % of the body weight; signs of slight pulmonary oedema were noted in 1 animal in the series. Ringer's solution was well tolerated and highly uraemic animals even improved after infusion of amounts equalling 15 % of their body weights; pulmonary oedema did not develop. Nor did pulmonary oedema develop even if the amount of Ringer's solution was increased to 50—100 % of the body weight in moderately uraemic animals, provided that the infusion was not given too rapidly. Alwall & Lindqvist also found that high salt and fluid retention only in rare cases caused slight pulmonary oedema in uraemic rabbits.

When the total amount of fluid was increased by infusion of urea or glucose solution, the animals died or be-

to establish the diagnosis of pulmonary oedema. Chest X ray gross observation, frothy fluid in the air ways, lung weight, water content of the lung and microscopical observation. In the vagotomy series supporting evidence by either microscopical findings or water content was required for the diagnosis of pulmonary oedema.

### Chapter 5 The control series

Alwall et al. (16) showed that rabbits whose ureters had been tied bilaterally lived for 1—6 days after the ureteral ligation without exhibiting any radiographic pulmonary changes in vivo and that their lung weights were microscopically normal post mortem. In the present studies (78 rabbits which after ureteral ligation had anuria for 0.1—4 days) it was noted in vivo that classical signs of pulmonary oedema (dyspnoea, rattling respiration, etc.) were not present, that the respiratory rate fell with increasing uraemia, that the heart rate remained unchanged, that the frequency of E.C.G. abnormalities, notably negative T waves, rose with increasing uraemia, that a slight cardiac enlargement occurred in about 15 % of severely uraemic animals and that slight radiographic pulmonary changes appeared within 3 days in 3 % of the animals.

Postmortally in animals killed by bloodletting, it was found that in most cases, even in severe uraemia, the macroscopical picture was normal, though in about 20 % there were a few to numerous punctiform haemorrhages and in about 3 % atelectatic

areas, that the amount of ascitic fluid increased markedly with increasing uraemia (averaging after 3—4 days 108 ml) whereas the amount of pleural effusion increased insignificantly and that frothy fluid in the air ways did not occur in uraemic animals. Measurements of the lung weight in 58 bled rabbits (weighing 1.38—5.87 kg) showed that the lung weight per kg body weight falls appreciably with increasing body weight. The normal value for the lung weight of rabbits is, according to Durlacher et al. (73)  $3.57 \pm 0.21$  g per kg. Luisada (154) quotes the figure  $4.55 \pm 0.21$  g per kg and Alwall et al. (21) 4.0 (3.2—5.0) g per kg body weight (the rabbits weighed 1.72—2.46 kg). In the present experiments the lung weights in 52 bled normal rabbits weighing 2.20—2.49 kg were  $4.17 \pm 0.53$  g per kg and in 78 bled uraemic (anuric for 0—4 days) animals  $4.25 \pm 0.45$  g per kg with no significant increase for the most severely uraemic animals. Alwall et al. (21) in 31 animals (weighing 1.53—2.50 kg) with anuria for 2 days, found an average lung weight of 4.11 (3.4—5.7) g per kg body weight. The water content in the lungs of 59 bled normal rabbits (weighing 1.38—5.87 kg) fell on an average insignificantly with increasing body weight. The normal value for rabbits is, according to Reichman (213) 77.1 % according to Mendenhall et al. (180) 79.8 % and according to Manery et al. (178) 80.2 % the latter two percentages are means, and the authors used freeze drying and drying in an oven, respectively. In the present experiments, dry

## A concise survey of literature and own experimental results

### Chapter 2 Literary survey on the problems of uraemic pulmonary oedema

The clinical, radiographic, and microscopical pictures of uraemic lung oedema are described. Special emphasis is placed on the difficulties attaching the differential diagnosis. The pathogenesis of uraemic pulmonary oedema is discussed. The role of fluid retention has been established (Alwall et al.) but its relation to many other possible factors such as uraemia, salt retention, the localisation of the fluid in the body, cardiac insufficiency, hypertension, hypoxia, anaemia, cerebral factors, and hypoproteinaemia has not been made clear.

### Chapter 3 Literary survey on experimental uraemic pulmonary oedema

Experimental pulmonary oedema in animals can be induced in several ways. The susceptibility to lung oedema varies from species to species. Experimental studies on uraemic pulmonary oedema, fluid lung and "fluid retention lung" are few. Only Alwall, Lunderquist et al., and Borgström et

al. have published investigations into this subject.

### Chapter 4 Technique

After preliminary trials, bloodletting was chosen as the method of sacrifice, for animals that did not die spontaneously. The experiments were made on rabbits and the following ten methods, the first four referring to *in vivo* observations, the rest to postmortem findings, were used in estimating the degree of pulmonary oedema: 1) Observations on the appearance and behaviour of the animals, 2) respiratory rate measurement, 3) electrocardiography (heart rate and E.C.G. disturbances), 4) chest X-ray, 5) gross observation, 6) observation and collection of frothy fluid in the airways postmortally, 7) measurement of lung weight absolutely and in relation to body weight, 8) estimation of water content in the lung, 9) microscopical observation, and 10) measurement of lung volume in a few experiments. The literature dealing with the technique used at these procedures is surveyed. In the present experiments a positive result of at least three of the following examinations was required.

changes were slight. As regards the respective criteria of pulmonary oedema, it may be noted that, for instance, gross haemorrhages in the lungs were seen in 88 % that a high water content was rare (1 case) with this form of overhydration, that chest radiography showed slight pulmonary changes, possibly ascribable to hyperaemia, in 33 % immediately after the transfusion and in 14 % immediately before the bloodletting, and that microscopic examination showed periarterial interstitial oedema in 2 cases and relatively large numbers of leucocytes in 4 cases.

#### *Increase of blood volume by dextran infusion*

Intravenous infusion of 2 ml of dextran solution per minute and kg body weight for about 2 hours did not produce pulmonary oedema in dogs (258). In the present study 6 % dextran solution 12 % of the body weight, was infused for 1 1/2 hours in 41 rabbits whose ureters had been tied bilaterally. Animals that did not die spontaneously were bled after 4—5 hours. Pulmonary oedema evidenced by three criteria developed in 2 out of 11 rabbits with anuria for less than 6 hours, in 6 out of 10 with anuria for 24 hours, in 7 out of 10 with anuria for 48 hours, and in 9 out of 10 with anuria for 72 hours. The frequency of pulmonary oedema increased significantly with increasing uraemia. After infusion of dextran in uraemic rabbits the frequency of pulmonary oedema was higher than after transfusion of the same amount of blood.

The six criteria of pulmonary oedema used here agreed less well in this series than in the others: the diagnosis of pulmonary oedema was made in 24 cases and was supported by all the six criteria in only 8 % and by five criteria in 43 %. The low percentages are due mainly to the failure of demonstrating alveolar oedema in 57 % of the 21 cases with at least four criteria of pulmonary oedema, and secondly to the fact that the water content was normal in 46 % of the cases with at least three criteria of pulmonary oedema. The great diagnostic difficulty in this series was to distinguish between pulmonary oedema and pulmonary haemorrhage, as haemorrhages of varying degrees were recorded in 98 % and less extent between pulmonary oedema and hyperaemia. It was therefore particularly difficult to establish the presence of pulmonary oedema on the basis of the gross picture and lung weight, but also radiologically.

#### *Increase of the extracellular fluid by infusion of saline*

The production of pulmonary oedema in rabbits by infusion of saline intravenously requires an infusion rate of at least 3 ml per minute and kg (53). Pulmonary oedema can be produced with a lower infusion rate if some other factor co-exists which in itself does not have a sufficient oedema-producing effect. (17 49 52 149 150). Fluid retention alone has not been found to produce pulmonary oedema in animals. Alwall et al. (17) infused

ing was done with a heat lamp and the water content was found to be  $81.0 \pm 0.9\%$  in normal animals and  $80 \pm 1.3\%$  in 78 uraemic (anuria for 0—4 days) animals. Microscopic examination showed no abnormalities in 12 rabbits with anuria for 3—4 days; focal haemorrhages in 3, one of which had atelectasis as well.

Durlacher et al. (73) found that congestion and oedema develop in the postmortem period in rabbits. I have examined 30 rabbits which died spontaneously in uraemia; the examination was carried out before rigor mortis had set in, 5—90 minutes after death. Frothy fluid was not found; the lung weight averaged  $4.98 \pm 0.64$  g per kg and the water content  $80.9 \pm 1.5\%$  (these figures were used as control values for spontaneously dead animals). Slight postmortal changes with hyperaemia and slight alveolar oedema were seen in 3 of the rabbits.

The postmortal changes were still more marked in animals that were examined 24 hours after death. In this group the lung weight was  $7.71 \pm 2.80$  g per kg body weight and the water content  $82.6 \pm 2.5\%$  (21 rabbits which died spontaneously in uraemia). Frothy fluid in air ways was noted in 19% (52 rabbits including 31 killed by injection of air). Gross observation showed either bright red light lungs or heavy dark red lungs; in some cases with mottled symmetrical markings towards the periphery which made it impossible to establish whether pulmonary oedema had developed *in vivo*. Microscopically there were profuse to moderate protein-rich type alveolar

oedema and hyperaemia, but red cells and alveolar phagocytes were rarely seen in the alveoli.

There was no significant difference in the lung weight or the water content between 16 normal and 15 severely uraemic rabbits 24 hours after they had been killed by intravenous injection of air.

Thus in these experiments no tendency towards pulmonary oedema could be demonstrated, neither in rabbits with increasing uraemia after ureteral ligation nor in rabbits that died in uraemia. Uraemic rabbits have no significant tendency to develop postmortal pulmonary oedema more frequently than normal rabbits.

## Chapter 6 Pulmonary oedema and overhydration

### *Increase of the blood volume by blood transfusion*

In dogs a blood transfusion amounting to 17% of the body weight for 7 hours does not necessarily produce lung oedema (129). In the present experiments, blood transfusions 12% of the body weights were given for  $1\frac{1}{2}$  hours intravenously to 21 rabbits whose ureters had been tied bilaterally, including 11 which had been anuric for a few hours and 10 with anuria for 48 hours. No reactions were noted *in vivo*. The animals were bled  $4\frac{1}{2}$  hours later. At least three criteria of pulmonary oedema were satisfied in 1 out of 11 rabbits with anuria for a few hours and in 2 out of 10 which had been anuric for 48 hours; the pulmonary

Thus, the toxicity of the mannitol solution rendered the assessment of its oedema producing effect difficult, but this effect seemed to be slight.

*Increase of all the fluid spaces by infusion of glucose solution*

Administration of 150—300 ml of 5 % glucose solution per kg and hour did not produce pulmonary oedema in dogs (257). Administration of large amounts of water through a gastric tube to rabbits produces fatal cerebral oedema (93). The present author infused 5.5 % glucose solution intravenously 15 % of the body weight, for 1½ hours in 28 rabbits whose ureters had been tied. As the blood sugar level became normal, signs of water intoxication appeared. Four rabbits died during the infusion, 9 were bled within 5—7 hours and 10 within 24 hours of the infusion. Five rabbits died spontaneously within 10—23 hours of the infusion. One rabbit which died during the infusion had slight pulmonary oedema the others did not have three criteria of pulmonary oedema.

*Increase of all the fluid spaces by infusion of urea solution*

Daily administration of 200 ml of 10—20 % urea solution intravenously to rabbits whose ureters had not been tied led to high concentration of urea and death of the animals after some time. Hypertension was noted in vivo and post mortem the lungs showed hyperaemia but no pulmonary oedema (235). Aqueous solutions of urea produce haemolysis, both in con-

centrated and in diluted solutions, whereas 25 % urea solution in, for instance isotonic glucose does not (218). The present experiments with infusion of urea solution comprise 42 rabbits with tied ureters. Isotonic (1.8 %) urea solution infused intravenously in amounts equalling 10—15 % of the body weight was toxic to uraemic rabbits which had been anuric for 48 hours, whether the solvent was water 5.5 % glucose, or 10 % invertose, and even when the infusion was given slowly by the subcutaneous route. Slightly uraemic rabbits, on the other hand, tolerated slow subcutaneous infusion of this amount of isotonic urea solution. Anuric rabbits died at an N.P.N. level of 300—400 mg per 100 ml, even when they were not overhydrated and the rise in N.P.N. was largely attributable to urea.

Pulmonary oedema as defined here occurred in 2 animals which were given 50 % urea solution by rapid intravenous infusion but did not occur in animals which were given isotonic urea solution or 30 % urea solution for 30 minutes.

## Chapter 7 Pulmonary oedema following anaesthesia induced with barbiturates

A technique similar to that described by Alwall (13 16) was used here.

Alwall used slow injection (20 minutes) of 1.2 ml of "Narkotal" solution per kg body weight. In the present experiments the dose was increased to 1.7 ml per kg body weight and deep narcosis was induced for



Ringer's solution, 12 % of the body weight in rabbits whose ureters had been ligated the animals survived 6 days after the infusion. Slight radiographic pulmonary changes occurred in 24 % of the cases but there was no clear cut case of pulmonary oedema. In 30 animals bled after 2 days of anuria the lung weight averaged 4.63 (3.2—6.39) kg (body weight 1.75—2.53 kg). With an increase of the dose to 25 % (Alwall et al [29]) the radiographic pulmonary changes increased to 60 % within 48 hours but no case of frank pulmonary oedema occurred and the series was complicated by a high frequency of profuse pleural effusion and atelectasis.

The present author has continued Alwall's et al experiments by giving infusions at varying times after the ureteral ligation. 65 rabbits in groups of 10—11 received infusions of 16 % Ringer's solution for 1 1/2 hours either 1/2 hour, 24, 48 or 72 hours after the ureteral ligation and were bled within 2—4 hours of the infusion or 48 or 72 hours after the ureteral ligation and were bled after 24 hours. One rabbit developed slight pulmonary oedema evidenced by three criteria: the animal was moribund and had severe E.C.G. changes before the infusion. The water content of the lung exceeded the upper limit of normal range in 13 % of the animals and slight radiographic pulmonary changes were seen in 6 %. No significant tendency to accumulation of fluid in the lungs could be noted in 62 bled uraemic rabbits: the lung weight averaged 4.39 (3.16—5.62) g per kg and the water content 82.2

(79.2—85.2) %. The administered fluid was partly deposited as ascites and pleural effusion. Improvement of general condition, fewer E.C.G. abnormalities and prolongation of the survival time were noted in severely uraemic rabbits after the infusion.

Massive slow infusion of Ringer's solution, 50—100 % of the body weight, produced atelectasis and no pulmonary oedema in 8 highly uraemic rabbits with tied ureters which survived the infusion.

#### *Increase of the extracellular fluid by infusion of mannitol solution*

Infusion of a solution of 5 % mannitol plus 5 % sorbitol in amounts equalling 10 % of the body weight did not produce pulmonary oedema in dogs (178). The present author infused isotonic (5.5 %) mannitol solution intravenously 15 % of the body weight for 5 hours in 20 rabbits with tied ureters and anuria for 48 hours (the planned infusion time of 1 1/2 hours had to be abandoned, as the animals died showing all the signs of cerebral oedema). After the infusion the animals were weak and their respirations slow. E.C.G. disturbances occurred in 63 % and cardiac enlargement in 47 %. The subcutis was rich in fluid and pleural effusion profuse (averaging 23 ml in bled animals). Only 3 rabbits survived 24 hours after the infusion. Pulmonary oedema evidenced by three criteria was present in 1 out of 10 bled rabbits but absent in 5 non rigid spontaneously dead rabbits. Pulmonary oedema of slight degrees could not be excluded in 5 rigid rabbits.

Thus, the toxicity of the mannitol solution rendered the assessment of its oedema producing effect difficult, but this effect seemed to be slight.

*Increase of all the fluid spaces by infusion of glucose solution*

Administration of 150—300 ml of 5 % glucose solution per kg and hour did not produce pulmonary oedema in dogs (257). Administration of large amounts of water through a gastric tube to rabbits produces fatal cerebral oedema (93). The present author infused 5.5 % glucose solution intravenously 15 % of the body weight, for  $1\frac{1}{2}$  hours in 28 rabbits whose ureters had been tied. As the blood sugar level became normal, signs of water intoxication appeared. Four rabbits died during the infusion, 9 were bled within 5—7 hours and 10 within 24 hours of the infusion. Five rabbits died spontaneously within 10—23 hours of the infusion. One rabbit which died during the infusion had slight pulmonary oedema the others did not have three criteria of pulmonary oedema.

*Increase of all the fluid spaces by infusion of urea solution*

Daily administration of 200 ml of 10—20 % urea solution intravenously to rabbits whose ureters had not been tied led to high concentration of urea and death of the animals after some time hypertension was noted *in vivo*, and post mortem the lungs showed hyperaemia but no pulmonary oedema (236). Aqueous solutions of urea produce haemolysis, both in con-

centrated and in diluted solutions, whereas 25 % urea solution in, for instance, isotonic glucose does not (218). The present experiments with infusion of urea solution comprise 42 rabbits with tied ureters. Isotonic (1.8 %) urea solution infused intravenously in amounts equalling 10—15 % of the body weight was toxic to uraemic rabbits which had been anuric for 48 hours, whether the solvent was water 5.5 % glucose, or 10 % invertose, and even when the infusion was given slowly by the subcutaneous route. Slightly uraemic rabbits on the other hand, tolerated slow subcutaneous infusion of this amount of isotonic urea solution. Anuric rabbits died at an N.P.N. level of 300—400 mg per 100 ml, even when they were not overhydrated and the rise in N.P.N. was largely attributable to urea.

Pulmonary oedema as defined here occurred in 2 animals which were given 30 % urea solution by rapid intravenous infusion but did not occur in animals which were given isotonic urea solution or 30 % urea solution for 30 minutes.

## Chapter 7 Pulmonary oedema following anaesthesia induced with barbiturates

A technique similar to that described by Alwall (13,16) was used here.

Alwall used slow injection (30 minutes) of 1.2 ml of 'Narkotal' solution per kg body weight. In the present experiments the dose was increased to 1.7 ml per kg body weight and deep narcosis was induced for

the purpose of obtaining more severe changes more quickly Alwall et al. performed ureteral ligation under Narkotal narcosis. I tied the rabbits ureters under local anaesthesia and did not give Narkotal narcosis until 2, 24, 48, or 72 hours after the ureteral ligation. Alwall et al. bled their animals after 48 hours or let them live until they died spontaneously after 1—5 days. I bled the animals that survived 24 hours after the termination of the experiment. Alwall et al. found radiographic pulmonary changes in 8% of non-overhydrated animals which had been subjected to ureteral ligation under Narkotal narcosis and allowed to live until they died spontaneously. I found such changes in 7% using a different narcosis technique and bloodletting after 24 hours in animals whose ureters had been tied. Alwall et al., in rabbits whose ureters had been tied under Narkotal narcosis and which were given an amount of Ringer's solution equalling 15% of the body weight for 1½ hours, noted radiographic pulmonary changes in one-third of the cases within 24 hours and in two-thirds during the observation period (1—5 days). In my experiments, using a different narcosis technique and bloodletting after 24 hours, I found radiographic pulmonary changes in one-third of the animals which had been subjected to ureteral ligation and overhydrated with Ringer's solution in amounts equalling 15% of the body weight for 1½ hours. In my series the lung weights were much higher. Alwall (13, 17), Alwall & Lunderquist (16) and Lunderquist (171) found that the radiographic pulmonary changes disappeared when the administered fluid was removed by ultrafiltration and concluded that fluid retention is a basic pathogenetic factor in pulmonary changes of the type discussed here (10). The present experiments were designed to study the importance of the uraemia factor in the frequency of pulmonary oedema following Narkotal narcosis in non-overhydrated and overhydrated animals with tied ureters.

In these experiments the ureteral ligation was performed under local anaesthesia (Xylocain) and narcosis was induced with Narkotal (1.7 ml per kg body weight) 2, 24, 48 and 72 hours after ureteral ligation. At least half the animals were given infusions of Ringer's solution (15% of the body weight for 1½ hours) immediately after completed injection of Narkotal. Those that lived 24 hours after the end of the experiment were bled. Three criteria of pulmonary oedema were not satisfied in any of the 10 rabbits that had received Narkotal narcosis after 2 hours of anuria nor in any of the 10 that were given the narcosis after 24 hours of anuria. Three criteria of pulmonary oedema were fulfilled in 2 of 10 rabbits given the narcosis after 48 hours of anuria and in 2 of 12 that received the narcosis after 72 hours of anuria.

Among the overhydrated animals at least three criteria of pulmonary oedema were satisfied in 1 of 22 with narcosis after 2 hours of anuria in 3 of 10 with anuria for 24 hours in 11 of 28 with anuria for 48 hours and in 10 of 22 with anuria for 72 hours. Pulmonary oedema developed in a greater number of cases among severely uraemic rabbits (anuria for at least 48 hours) than among slightly uraemic rabbits (anuria for less than 12 hours). In the series of overhydrated rabbits this difference was significant. Pulmonary oedema following Narkotal narcosis developed in a greater number of cases among rabbits with salt and fluid retention than among rabbits with tied ureters without salt and

fluid retention in all stages of uraemia but the difference between the two groups was greatest in severely uraemic rabbits ( $p=0.00$ )

Bronchiolitis occurred in half the rabbits after 'Narkotal' narcosis. The duration and the depth of narcosis do not seem to have influenced the frequency of pulmonary oedema. The highly uraemic animals were, however more markedly affected by the narcosis than were slightly uraemic animals, which may have been of significance as regards the frequency of pulmonary oedema. In this series, lung oedema occurred in mild degrees here and there in the hilar or basal regions and in such cases the diagnosis rested mainly on the gross and the microscopical findings.

## Chapter 8 Pulmonary oedema following injection of adrenaline

A technique similar to that described by Drenkhahn (69) was used in producing pulmonary oedema in rabbits.

The adrenaline dose used by Drenkhahn, 3 mg per kg body weight, was in this series reduced to 1 mg per kg in view of the other experimental conditions. The latter dose caused within 20 minutes pulmonary oedema as defined here in 40 % of a control series of 10 animals. The rest of the experiments were made on rabbits whose ureters had been tied. The experiments reported in the literature do not seem to have been carried out under conditions that were similar to those used here.

Adrenaline was injected intramuscularly in non-overhydrated and overhydrated rabbits whose ureters had been tied 1—4, 48, and 72 hours, respectively before the injection. Each group comprised 10 rabbits. Among these animals, which were thus slightly to severely uraemic, pulmonary oedema developed in the non-overhydrated series in 50 % 40 % and 30 % of the respective group. In the control series the corresponding figure was 60 %. Among animals which were overhydrated by infusion of Ringer's solution in amounts equalling 15 % of their body weights, pulmonary oedema evidenced by at least three criteria was noted in 60 % 50 % and 50 % respectively. Accordingly the frequency of adrenaline-induced pulmonary oedema did not increase with increasing uraemia under these experimental conditions.

However in the adrenaline series the number of rabbits that died without having pulmonary oedema was higher among severely uraemic (anuria for at least 72 hours) than among the less severely uraemic (anuria for less than 72 hours) animals, or 55 % as against 16 %. The explanation of this may be that in highly uraemic animals death was more often due to some adrenaline effect other than pulmonary oedema. In interpreting the results of the adrenaline series it was therefore difficult to determine whether severe uraemia leads to increased tendency towards pulmonary oedema.

The frequency of pulmonary oedema was slightly higher among overhydrated than among non-overhydrated

ed animals but the difference is not statistically significant. It was difficult to render the two groups comparable, for several reasons. The concentration and amount of adrenaline may have been different between the groups, in that the overhydrated animals had a larger fluid space ( $> 15\%$ ) and were given a higher dose ( $15\%$ ) of adrenaline than the non-overhydrated animals and the rate of absorption for the intramuscular adrenaline could have differed between the two groups.

This series illustrates the difficulties of obtaining comparable groups for the purpose of throwing light on the problems studied by these experiments.

## Chapter 9 Pulmonary oedema following vagotomy

The variegated microscopical picture of pulmonary changes following vagotomy has been described by earlier authors (168 213 246). Kraus (150) found that infusion of saline after vagotomy produces pulmonary oedema in rabbits. Brunn's (42) brief report that nephrectomy prevents the development of pulmonary oedema after subsequent vagotomy and overhydration was not verified by the experiments on rabbits with tied ureters described below. Experiments in animals with tied ureters do not seem to have been reported in the literature. No previous author has given the whole infusion before vagotomy. The risk of asphyxia in handling the animals after vagotomy must be considered.

61 rabbits were subjected to bilateral

ureteral ligation, but the series was reduced to 57 since 4 rabbits were found dead after rigor mortis had already set in and were therefore excluded. The animals were vagotomized 0—48 hours after the ureteral ligation. Immediately before the vagotomy half the animals were given infusions of Ringer's solution in amounts equalling  $15\%$  of their body weights for  $1\frac{1}{2}$  hours. Among 29 non-overhydrated animals 15 were submitted to vagotomy within 0—4 hours of ureteral ligation and 14 within 48 hours of ureteral ligation. In 28 overhydrated animals vagotomy was performed in 14 within 3—5 hours and in 14 within 48 hours of the ureteral ligation. Those that did not die spontaneously were killed by bloodletting within about  $14\frac{1}{2}$  hours of the vagotomy. 4 moribund animals were bled within only 5—10 hours.

The pulmonary changes following vagotomy consisted of mixed oedema, pneumonia, atelectasis, hyperaemia and haemorrhages in various combinations. The method for establishing pulmonary oedema used in the other series, namely on the basis of at least three criteria, proved unsatisfactory in the vagotomy series. Seven rabbits with outspoken pneumonia came to be included in the pulmonary-oedema group. The requirements for the diagnosis of pulmonary oedema therefore changed so that the three criteria to be satisfied were to include microscopical evidence of alveolar oedema or a water content exceeding the upper limit of normal range. Arguments in favour of this method of assessment are that

extensive pneumonia is readily diagnosed microscopically and that the water content can be presumed to be normal in cases of pneumonia (see Poulsen [211] and Diagram 17)

23 rabbits had pulmonary oedema according to this definition. In 15 of them the diagnosis was supported by at least five criteria. Among 24 rabbits without pulmonary according to this diagnosis, 80 % (27 rabbits) had at most two positive criteria, but in the intermediate group (26 % of all the rabbits) the diagnosis was difficult. In this series great uncertainty attached to all the criteria of pulmonary oedema used here.

Pulmonary oedema according to this definition was in the non-overhydrated series diagnosed in 2 out of 15 slightly uraemic animals and in 5 out of 14 severely uraemic animals. In the overhydrated series it was established in 9 out of 14 slightly uraemic and 7 out of 14 severely uraemic animals. Thus, the frequency of pulmonary oedema was higher in cases of severe

than in cases of slight uraemia among non-overhydrated animals and the reverse in overhydrated animals. The difference in this respect between slightly uraemic and highly uraemic animals is not statistically significant in either series. The frequency of pulmonary oedema was higher among overhydrated than among non-overhydrated rabbits, notably in cases of slight uraemia for which the difference is almost significant ( $0.01 < p < 0.02$ )

Another difficulty was that vagotomy lung oedema takes some time to develop. Five animals died in asphyxia so soon after the vagotomy that there was reason to suspect that pulmonary oedema had not had time to develop.

The problems that arose in this series may be of special interest, since a parallel can be drawn to clinical conditions where the differential diagnosis against pulmonary oedema presents difficulties in the presence of pneumonia, haemorrhages, and atelectasis (see Alwall [20-21])

## Summary

In a survey of 903 patients with acute and chronic renal failure, Alwall (21) showed that the frequency of the uraemic pulmonary oedema is high (42.7 %) and emphasized that these pulmonary changes are of great importance in the diagnosis and treatment of cases in a renal medical clinic. The difficulties of making a correct diagnosis in vivo of uraemic pulmonary oedema were also discussed.

I have tried to elucidate the diagnostic and pathogenetic problems of uraemic pulmonary oedema by experimental studies in animals.

### *Detection and quantitative study of pulmonary oedema*

In this study six methods were used for the evaluation of pulmonary oedema. Chest X-ray, gross observation, observation and collection of frothy fluid in the air ways, measurements of lung weight, analysis for percentage water content in the lungs, and microscopic observation, and the results of these methods were compared. If it was supported by at least three of these criteria the diagnosis of pulmonary oedema was established. In the vagotomy series, the three criteria

to be satisfied had to include microscopical lung oedema or a raised water content. The appearance and behaviour of the animals, respiratory rate, E.C.G. and heart rate, and in some experiments the lung volume were also recorded. After ligation of the ureters in uraemic rabbits pulmonary oedema was induced by hypervolaemia (blood, dextran), vasoactive drugs (adrenaline), pharmacological agents (barbiturates) and alteration of the peripheral nervous and respiratory systems (vagotomy). About half the animals were overhydrated with Ringer's solution; the degree of uraemia varied from insignificant to severe. All these methods were used because the writer wished to study the importance of the uraemia factor and the salt fluid retention factor and to evaluate different criteria of pulmonary oedema under varying experimental conditions. In only a few experiments was sudden pulmonary oedema induced. The object was in most cases to induce slowly supervening pulmonary oedema.

*The appearance and behaviour of the rabbits.* The observation of the animals' appearance and behaviour was valuable, particularly in cases of acute

sudden pulmonary oedema, but the assessment became increasingly hazardous the weaker the animal's power of reaction and the greater the number of complications that occurred.

*Respiratory rate* The respiratory rate rose in association with e.g. restlessness, lung oedema, and atelectasis, and fell at rest, with increasing uraemia, after vagotomy, narcosis, and pre-mortally as well as in the supine position. Normal restless animals could have an equally high respiratory rate as animals with pulmonary oedema. On an average, the rate was higher in those with lung oedema, even the severely uraemic ones.

*E.C.G. and heart rate* Most rabbits with E.C.G. disturbances had no pulmonary oedema and many rabbits with pulmonary oedema had only slight E.C.G. changes. The heart rate also proved to be of little diagnostic value, as it changed insignificantly in association with pulmonary oedema and uraemia.

*Chest X ray* X ray density changes could be detected even in cases of slight other criteria of pulmonary oedema. In most cases the X ray evidence was well consistent with the other criteria of pulmonary oedema. The situations in which the X ray did not agree with other criteria were acute sudden pulmonary oedema (rapid injection of barbiturates intravenously and drenaline intramuscularly) pulmonary oedema with basal involvement or in the form of numerous small

circumscribed lesions (the Narkotal and the dextran series) in which very little was visible on the film, pneumonia (the vagotomy series) haemorrhages into the lung parenchyma (the dextran and the Narkotal series) atelectasis (the vagotomy series, overhydrated animals) and possibly hyperaemia (overhydrated animals) in which differential diagnosis was difficult. Technical factors were a minor problem. Chest radiography is useful in experiments *in vivo*, as in Alwall's and Linderquist's studies (13, 16, 17 21 38 171 a).

*Gross observation.* It was the most sensitive of all the methods for diagnosis of experimental pulmonary oedema but also subjected to great uncertainty as regards slight pulmonary changes. With the macroscopical evaluation used in this study pulmonary oedema evidenced by this criteria was diagnosed in excess. Gross observation is useful as a quick method which is particularly expedient in detecting all forms of local pulmonary changes. Its inadequacy relates to the subjective gradation and the differential-diagnostic difficulties that arise in cases of co-existent severe hyperaemia haemorrhages into the lung parenchyma pneumonia, atelectasis, and postmortal oedema.

*Frothy fluid in the air-ways* The method was found to be a simple and quick aid to the diagnosis of marked pulmonary oedema. Many animals with slight pulmonary oedema or in poor general condition had no frothy



fluid animals with profuse thin oedema fluid had very little frothy fluid, as had those with viscid fibrinous oedema fluid. It was often difficult to distinguish between bronchitis stagnation of secretion and haemorrhages into the respiratory tract and lung oedema secretion

*Lung weight* Pneumonia hyperaemia and pulmonary haemorrhages caused an increase of the lung weight but atelectasis did not and animals with white spotted lungs which occurred after overhydration did not have increased lung weights

Only some of those animals that died in uraemia or were killed by intravenous injection of air had increased lung weights after death. The position of the body, hyperaemia at the moment of death, overhydration, postmortal pressure in the blood vessels, and clotting time may possibly play a part. It was therefore difficult to state normal values for the lung weight in spontaneously dead animals, especially for those that were not examined immediately after death. In association with experimental pulmonary oedema without any noteworthy pneumonia, hyperaemia, pulmonary haemorrhages or postmortal changes (this refers only to non bled animals) the lung weight is a good method for evaluation of the oedema state. The presence of pulmonary oedema can be excluded if the postmortal lung weight is low

*The water content of the lung* In this study the lungs were dried by the heat

lamp technique, which has not been used earlier for this purpose. In animals with haemorrhagic pulmonary oedema or with pulmonary oedema and marked hyperaemia or pneumonia the water content was in some cases normal although the lung weight, gross and microscopical observations, X ray and the presence of frothy fluid provided evidence of pulmonary oedema. It was difficult to establish basic values for overhydrated animals without the diagnosis of pulmonary oedema many of them had a water content that exceeded the upper limit of normal range but no other criteria of pulmonary oedema. In spontaneously dead rabbits the water content increased less than the lung weight post mortem except in animals that died markedly overhydrated. Small foci of pulmonary oedema as in the Narkotal series were not evidenced by an increased water content. The method is particularly valuable in the differential diagnosis preferably together with the lung weight, as has been shown by Poulsen (206). In complicated cases further criteria of pulmonary oedema must be satisfied before the diagnosis of pulmonary oedema can be established with a reasonable degree of probability

*Microscopical studies* There was no alveolar oedema in lungs with massive oedema evidenced by all the other criteria notably in the dextran series and in overhydrated rabbits in the adrenaline and the vagotomy series. Microscopical examination was necessary as a means of making the differ

ential diagnosis, especially in the vagotomy series in which some animals had co-existing pulmonary oedema, pneumonia, atelectasis, hyperaemia, and haemorrhages. The diagnosis and quantitation of alveolar oedema of lung-oedema origin was also difficult in the vagotomy "arkofal" and dex tran series, in which alveolar oedema was seen around numerous small areas of haemorrhage and infection.

Only the most severely changed part of the lungs were examined microscopically which means that the evaluation refers to local amounts and is not a general quantitation. In post mortal pulmonary oedema the alveolar oedema fluid was rich in protein, few alveoli contained red cells and alveolar phagocytes, and even peripheral alveoli were well filled.

Microscopical observation is of value in the differential diagnosis rather than in the diagnosis and quantitation of pulmonary oedema.

*Lung volume* The lung volume was determined in a small number of rabbits. The method was abandoned when it was found that great variations were obtained between the volumes because the air disappeared very quickly from the lung at the least leakage, which occurred easily.

The difficulties in establishing which animals have and which have not pulmonary oedema seem to be due to three factors. There is no available diagnostic method which is suitable under any conditions, the borderlines are vague, many pulmonary processes can co-exist with pulmonary oedema. It is not possible to choose the best

method, because the results vary with different forms of lung oedema. On the basis of the observations made in these experiments the range of usefulness of the methods under discussion is outlined.

When three of the six criteria used here are taken as sufficient evidence for pulmonary oedema it may be suspected that pulmonary oedema would be wrongly diagnosed in pneumonia, hyperaemia, and haemorrhages into the lung parenchyma. Moreover quite dissimilar microscopical pictures may be obtained at different combinations of three positive criteria. One rabbit can, for instance have slight uncomplicated pulmonary oedema while another can have pulmonary oedema with evidence of haemorrhage. The problem arising in haemorrhages or hyperaemia seems to be a minor one, as abundant haemorrhages are closely associated with haemorrhagic pulmonary oedema, and marked hyperaemia is closely associated with pulmonary oedema. In pneumonia the method failed, however. Here, microscopical examination or analysis of the water content was necessary to avoid designating pneumonia as pulmonary oedema.

Poulsen (206) has suggested a combination of water content and lung weight as a means of evaluating and diagnosing pulmonary oedema. This method was tried in the present study and showed good agreement in most cases. The doubtful cases are those in which the presence of both pulmonary oedema and pneumonia (vagotomized animals) pulmonary oedema and hy

peracmia and pulmonary oedema and haemorrhages into the lung parenchyma (the dextran series) is suspected. In these cases other examinations in addition to lung weight and water content are necessary, notably microscopic observation in order to establish a reasonably confident diagnosis.

These diagnostic difficulties arise not only in experimentally induced pulmonary oedema but also and perhaps more emphatically in clinically manifest pulmonary oedema. It will be seen in Chapter 2 that clinically acute violent frothy lung oedema is easy to diagnose whereas slowly supervening pulmonary oedema may present great diagnostic difficulties. Cases with bronchitis, bronchopneumonia, atelectasis and/or haemorrhages, in particular are difficult to distinguish from cases with pulmonary oedema, and the borderline between hyperaemia and pulmonary oedema is vague.

In several cases pneumonia or bleeding in the lung parenchyma led to the radiographic diagnosis of fluid retention lung and therapy was thus inadequate. Long standing heart failure with pulmonary congestion can render the radiographic differential diagnosis difficult. Chest radiograph has its greatest value in the diagnosis of "fluid retention lung" (uraemic pulmonary oedema) under clinical conditions where overhydration is the main alternative and pneumonia etc. appears less probable (Alwall 1963). The frequency of postmortal pulmonary oedema is high among uraemia patients whether it is final or postmortal is an open question.

### *The uremia factor and the fluid factor*

Under these experimental conditions, uremia per se seems not to be sufficient to cause pulmonary oedema; other factors must be present.

To find out whether increasing uraemia increases the susceptibility to pulmonary oedema, experimental pulmonary oedema was induced by four methods in rabbits which had been submitted to bilateral ureteral ligation. Increasing uraemia increased the tendency to pulmonary oedema after infusion of dextran and after Narkotal narcosis, whether the animals were overhydrated or not; this effect of uraemia was insignificant in non-overhydrated vagotomized rabbits and absent after injection of adrenaline, in overhydrated as well as in non-overhydrated animals. Perhaps severe uraemia increases the susceptibility to pulmonary oedema under certain conditions only or else—hypothetically—some factors of the uraemia phenomena predispose to lung oedema while others counter it (Ascites can be such a factor). Unspecific factors in the experimental conditions can be thought to influence the frequency of pulmonary oedema under otherwise similar conditions.

On the basis of these considerations it may be concluded that uraemia following ligation of the ureters can be a factor that under some conditions increases the susceptibility to pulmonary oedema and under other conditions reduces it.

The significance of the fluid factor

was studied by overhydration experiments on rabbits whose ureters had been tied, using different solutions for infusion, as well as by overhydration with Ringer's solution in connection with vagotomy. Narkotal narcosis, and adrenaline injection. Salt and fluid retention increased the susceptibility to pulmonary oedema after 'Narkotal' narcosis, adrenaline injection, and vagotomy in anuric rabbits. (Note worthy in these series was the presence of bronchiolitis after 'Narkotal' narcosis, periarterial interstitial oedema after adrenaline injection and mixed oedema pneumonia, atelectasis, hyperaemia and haemorrhages after vagotomy) Even after administration of blood in amounts that were twice as large as those of the animals circulating blood, slight pulmonary changes developed in only a few cases. But when the plasma volume was increased by the addition of dextran, pulmonary oedema developed in a higher frequency than after the same amount of blood. When the extracellular fluid was increased by infusion of mannitol solution, signs of slight pulmonary oedema arose in only 1 case. An increase by infusion of Ringer's solution did not cause pulmonary oedema even 1 administration of amounts equalling 50—100 % of the body weight, unless the infusion was

made too rapidly and the animal was moribund before the infusion. When the total fluid was increased by infusion of urea or glucose solution, the animals became very poor but did not develop pulmonary oedema. The toxicity of urea and mannitol solutions rendered the assessment of their oedema producing effect difficult, but this effect seemed to be slight.

The main conclusion that can be drawn from these experiments is that overhydration in association with uraemia in rabbits with both their ureters tied produced pulmonary oedema only if it meant an increase of the blood volume. Additional factors are evidently required in order that overhydration by infusion of other solutions shall produce pulmonary oedema. Salt and fluid retention increased the susceptibility to pulmonary oedema after 'Narkotal' narcosis, adrenaline injection, and vagotomy in anuric rabbits.

The problems that arose in these series may be of special interest, since a parallel can be drawn to clinical conditions, where the differential diagnosis against pulmonary oedema presents difficulties in the presence of pneumonia haemorrhages, and atelectasis, etc. (see Alwall [20-21])

## Acknowledgements

I wish to express my sincere gratitude to Professor Nils Alwall for awakening my interest in problems on uraemic pulmonary oedema for excellent working facilities, for all the support he has given throughout the present investigation and for constructive criticism

Professor Olle Olsson for help with some of the roentgenological facilities

Nils O Berg Prosector at the Institute of Pathology for generous help and examinations of the histological preparations

Dr Anders Lunderquist for generous help and examination of the X-ray pictures

Göran Angamark, fil. kand., the Institute of Statistics, for help with the statistical calculations

Mrs. Olga Friedlitz for skilful technical assistance Mrs. Maria Roth for conscientious secretarial work, Karl Råhesoo Pharm. D and Mr Jozsef Szalai, for careful work with the diagrams, Miss Mona Frisell for the histological preparations, Miss Ing Britt Augrell for the photographs, Bozyslaw Kurowski, jur mag Miss Judith Csöreg, Mrs. Maria Kurowska for technical assistance, Miss Elsa Schmitz for helping with proof reading and Mr A. Nilsson for good care of the animals.

The investigation was supported by grants from the Medical Faculty of the University at Lund Sweden and (through professor Nils Alwall) from the Swedish Medical Research Council and Marie-Louise Schéle's Memorial Fund.

# References

1. *Altman, J. K.* Fluid volumes and electrolyte concentrations in normal rabbits. *Amer. J. Physiol.* 127 860, 1950.
2. *Alexander, S., Ent, A., J. C., Freedman, H. J.* Some experimental observations on the action of intravenous hypertonic urea in dogs, with particular reference to plasma volume and tissue water changes. *J. Neurol. Neurosurg. Psychiat.* 24 148, 1961.
3. *Altchek, M. D.* Acute pulmonary edema. Grune & Stratton, New York, 1954.
4. *Altchek, M. D., Gilligan, H. R.* The effects on the cardiovascular system of fluids administered intra-cranially in man. II. The dynamics of the circulation. *J. clin. invest.* 37 401 1958.
5. *Almoff, N.* On the artificial kidney I. Apparatus for dialysis of blood in vivo. *Acta med. scand.* 758 317 1947.
6. *Almoff, N.* Treatment of acute renal failure with anuria-oliguria. The importance of weight control and the hazards of excessive electrolyte-fluid supply. *Scand. Med.* 40 2378, 1948.
7. *Almoff, N.* On the artificial kidney II. Some clinical experiences and problems in the treatment of uremia. *Nord. Med.* 41 411 1949.
8. *Almoff, N., Bergsten, B., Gella, P., Norrby, L., Ström, A.* On the artificial kidney IV (Technique in animal experiments) *Acta med. scand.* 157 302, 1949.
9. *Almoff, N., Norrby, L., Ström, A.* On the artificial kidney V. Some experiments during the study of dialytic treatment on animals with uremia caused by mercuric chloride poisoning. *Acta med. scand.* 152 477 1949.
10. *Almoff, N., Hjerpe, B.* On the artificial kidney VI. Some views on the indications for treatment of uremia and for active removal of oedema by means of our artificial kidney based on studies of uremic material not treated with this method. *Acta med. scand.* 152 572, 1949.
11. *Almoff, N.* On the artificial kidney VIII. Cases of acute renal failure with anuria-oliguria treated with (1) Excessive fluid-electrolyte supply (2) Fluid supply under weight control to avoid or reduce fluid retention-oedema (3) Artificial kidney. *Acta med. scand.* Suppl. 229 1, 1949.
12. *Almoff, N.* On the artificial kidney XIII. Construction details of the dialyser-citrafiltrator intended for home. *Acta med. scand.* Suppl. 229 30 1949.
13. *Almoff, N.* Treatment of uremia, anuric poisoning and life-threatening electrolyte-fluid retention, especially by means of artificial kidney. *Urologia (Milano)* 2: 482, 1952.
14. *Almoff, N., Linderquist, A., Tornberg, A.* Four cases of acute glomerulonephritis anuric for 12, 11, 12 and 11 days respectively. *Nord. Med.* 43, 1565 1952.
15. *Almoff, N.* A weighing machine for bed patient. An indispensable item of equipment for every hospital. *Acta med. scand.* 143 188, 1952.
16. *Almoff, N., Linderquist, A., Olsson, O.* Studies on electrolyte-fluid retention. I. Uremic lung-fluid lung? On pathogenesis and therapy (A preliminary report.) *Acta med. scand.* 146 187 1953.

## Acknowledgements

I wish to express my sincere gratitude to

Professor Nils Alwall for awakening my interest in problems on "uraemic pulmonary oedema" for excellent working facilities, for all the support he has given throughout the present investigation and for constructive criticism

Professor Olle Olsson for help with some of the roentgenological facilities

Nils O Berg Prosector at the Institute of Pathology for generous help and examinations of the histological preparations

Dr Anders Lunderquist for generous help and examination of the X ray pictures

Göran Angamark M. kand., the Institute of Statistics for help with the statistical calculations

Mrs. Ilga Friedlits for skilful technical assistance, Mrs. Maria Roth for conscientious secretarial work, Karl Råhesoo, Pharm D and Mr Jozsef Szalai for careful work with the diagrams, Miss Mona Frisell for the histological preparations, Miss Ing Britt Augrell for the photographs, Bozyslaw Kurowski jar mag Miss Judith Csöreg, Mrs. Maria Kurowska for technical assistance, Miss Elsa Schmitz for helping with proof reading and Mr A. Nilsson for good care of the animals

The investigation was supported by grants from the Medical Faculty of the University at Lund Sweden and (through professor Nils Alwall) from the Swedish Medical Research Council and Marie-Louise Schéle's Memorial Fund.

## References

1. *Alm, J. E.* Fluid volumes and electrolyte concentrations in normal rabbits. *Amer J Physiol* 162 685, 1950.
2. *Alexander, S., Eaton, J. C., Freedman, H. J.* Some experimental observations on the action of intravenous hypertonic urea in dogs, with particular reference to plasma volume and tissue water changes. *J Neurol. Neurosurg. Psychiat.* 23 148, 1961.
3. *Altshuler, M. D.* Acute pulmonary edema. Grune & Stratton, New York, 1954.
4. *Altshuler, M. D., Gibbons, H. R.* The effects on the cardiovascular system of fluids administered intra-venously in man. II The dynamics of the circulation. *J clin. invest.* 17 491 1938.
5. *Alwall, V.* On the artificial kidney I. Apparatus for dialysis of blood *in vitro*. *Acta med. scand.* 723 217 1947.
6. *Alwall, V.* Treatment of acute renal failure with anuria-oliguria. The importance of weight control and the hazards of excess electrolyte-fluid supply. *Nord. Med.* 40 2378, 1948.
7. *Alwall, V.* On the artificial kidney II. Some clinical experiences and problems in the treatment of uraemia. *Nord. Med.* 41 411, 1949.
8. *Alwall, V., Bergsten, B., Grönlund, P., Norström, L., Sjöström, A.* On the artificial kidney III (Technique in animal experiments). *Acta med. scand.* 132 303, 1949.
9. *Alwall, V., Norström, L., Sjöström, A.* On the artificial kidney V. Some experiences during the study of dialytic treatment on animals with uraemia caused by urethane chloride poisoning. *Acta med. scand.* 132 477 1949.
10. *Alwall, V., Harnær, E.* On the artificial kidney VI. Some laws on the indications for treatment of uraemia and for active removal of oedema by means of our artificial kidney based on studies of anemic material not treated with this method. *Acta med. scand.* 132: 575, 1949.
11. *Alwall, V.* On the artificial kidney VIII. Cases of acute renal failure with uraemia-oliguria treated with (1) Excessive fluid-electrolyte supply (2) Fluid supply under weight control to avoid or reduce fluid retention-oedema. (3) Artificial kidney. *Acta med. scand. Suppl.* 229 I 1949.
12. *Alwall, V.* On the artificial kidney XIII. Construction details of the dialyser-ultrafilter intended for humans. *Acta med. scand. Suppl.* 229 30 1949.
13. *Alwall, V.* Treatment of uraemia, anuric poisoning and life threatening electrolyte-fluid retention, especially by means of artificial kidney. *Urologia (Milano)* 2 483 1952.
14. *Alwall, V., Linderqvist, A., Törnberg, A.* Four cases of acute glomerulonephritis treated for 14, 21, 33 and 44 days respectively. *Nord. Med.* 48: 1863, 1952.
15. *Alwall, V.* A weighing machine for bed patients. An indispensable item of equipment for every hospital. *Acta med. scand.* 148 188, 1952.
16. *Alwall, V., Linderqvist, A., Olsson, U.* Studies on electrolyte-fluid retention. I. Uraemia lung-fluid lung? On pathogenesis and therapy (A preliminary report). *Acta med. scand.* 148 187 1952.



## Acknowledgements

I wish to express my sincere gratitude to Professor Nils Alwall for awakening my interest in problems on "uraemic pulmonary oedema" for excellent working facilities for all the support he has given throughout the present investigation and for constructive criticism

Professor Olle Olsson for help with some of the roentgenological facilities

Nils O Berg Prosector at the Institute of Pathology for generous help and examinations of the histological preparations

Dr Anders Lunderquist for generous help and examination of the X-ray pictures

Göran Angsmark fil. kand., the Institute of Statistics, for help with the statistical calculations

Mrs. Ilga Friedlits for skilful technical assistance Mrs. Maria Roth for conscientious secretarial work, Karl Råhesoo Pharm D and Mr Jozsef Szalai for careful work with the diagrams, Miss Mona Frisell for the histological preparations, Miss Ing Britt Augrell for the photographs, Bozyslaw Kurowaki, jur mag Miss Judith Csöreg Mrs. Maria Kurowaka for technical assistance, Miss Elsa Schmitz for helping with proof reading and Mr A Nilsson for good care of the animals

The investigation was supported by grants from the Medical Faculty of the University at Lund, Sweden and (through professor Nils Alwall) from the Swedish Medical Research Council and Marie-Louise Schéles Memorial Fund

43. Carr C. J. Mayer R. Schmidt J. E., Kram J. C. The fate of mannitol and mannitol in the animal body. *J. Biol. Chem.* 102 721 1933.
44. Chesley K. K. Studies on the mechanism of adrenaline lung oedema. *J. Path. Bact.* 76 211 1938.
45. Cohnheim, J. *Lichtheim, L.* Chem. hyd. 2. mal und hydrinischer Oedem. *Arch. f. path. Anat. und Phys.* 69 106, 1877
46. Coeurtice F. C. Pulmonary oedema. Clinical implications of laboratory experiments. *Aust. N. Z. J. Surg.* 22 177 1933
47. Coeurtice F. C., Kerner P. I. The effect of sodium on pulmonary oedema produced by massive intravenous infusions. *Aust. J. exp. Biol. med. Sci.* 30 811 1932.
48. Cressy C. H. Observations on hemolysis during transurethral resection. The effects of urea. *Trans. Amer. Ass. gen. urin. Surg.* 47 8, 1931
49. Cwiling O. Varpe, L., Sæm I. Die röntgenologische Darstellung des experimentellen Lungenödems. *Z. Kreisf. Forsch.* 48 990 1960.
50. Deamer R. L., Col N. N. V. Lung an experimental study. *Ann. Surg.* 137 836, 1945.
51. Dreire M. Leye P. Le la sign du sang. *Arch. de physiol. norm. et path.* p. 63, 1888
52. Demer ( E. Heart failure in acute nephritis. *Quart. J. Med.* 78 163, 1931
53. Dugl J. T. W. Lamm, J. S., Hal A. E. H. Warren, J. I. The effect of intra venous inf. of physologic saline solution on the pulmonary arterial and pulmonary capillary pressure in man. *J. clin. Invest.* 30 343, 1951
54. De Fazio V., Christensen, R. C. Rogers, T. J. Doe J. J. Verdon, F. Hellme H. A. Circulatory changes in acute glomerulonephritis. *Circulation*, 30 190, 1933.
55. De Garm, E. L. Gross sequelae of blood transfusions. A clinical study of 13 cases occurring to 1500 blood transfusions. *Ann. Intern. Med.* 11 1 77 1938
56. De Garm E. L. Hardin, R. C. Mosper J. R. Blood transfusions. W. B. Saunders Co Philadelphia, London 1939
57. De Greeff J. d. Boun, P. J. Bilateral renal cortical necrosis. *Acta med. scand.* 163 341, 1939
58. De Pass S. W. Stein, J. Poppel, M. H. Pulmonary congestion and edema in uremia. *J. Amer. med. Ass.* 167 4, 1936.
59. Deck, H. J. The evil sequelae of completed rest. *J. Amer. med. Ass.* 175 1833, 1944.
60. Domínguez R., Carcamo, A. C., Page I. H. Mannitol. Kinetics of distribution, excretion and utilization in human beings. *J. Lab. clin. Med.* 57 1192, 1947
61. Dorsch, J. Uremic edema of the lungs. *Am. J. Roentgenol.* 53 670, 1917
62. Dorsch, J. Uremic edema of the lungs. *Lancet* 2 911 1919
63. Dorsch J. Morrison, B., Steiner R. E. Lung changes during hexamethonium therapy for hypertension. *Brit. Heart J.* 16 161 1934
64. Dorland's Illustrated Medical Dictionary W. B. Saunders Company Philadelphia and London, 1937
65. Dwyer J. W. The problem of overtransfusion in massive hemorrhage. *Ann. Surg.* 148 73, 1936.
66. Drzewilka, F. O. Die Beziehung zwischen Pulmonalvenendruck und Ödementstehung beim experimentiellen Adrenallungenödem. *Verh. dtsch. Ges. Kreisf. Forsch.* 23 372, 1937
67. Drzewilka, F. H. Die Ödementstehung beim experimentiellen Adrenallungenödem. *Pflügers Arch. f. Physiol.* 295 231 1968
68. Drinker C. K. Pulmonary edema and inflammation. *Harvard Lab. Press. Cambridge Mass.* 1947
69. Drinker C. K. Warren, M. F. The genesis and resolution of pulmonary transudates and exudates. *J. Amer. med. Ass.* 172 360 1953.
70. Dundee J. W. Richards, R. R. Effect of azotemia upon the action of intra venous barbiturate anesthesia. *Anesthesiology* 13 333, 1934
71. Dwyer S. H. Banfield W. C. Byrnes J. D. Post-mortem pulmonary edema. *Yale J. Biol. Med.* 22 543, 1930.

- 17 Alwall V Treatment of electrolyte-fluid retention by ultrafiltration of the blood in vivo The Kidney Club Foundation, London, p 221 1954
- 18 Alwall V. Ergebnisse von 700 Behandlungen mit der Künstlichen Niere (Dialyse Ultrafiltration) während der Jahre 1946—1953 sowie Mobilisierung, Tracheotomie, Respirationsbehandlung u.s.w. und röntgenologische Kontrolle der Flüssigkeitsgleichgewichte. Dtsch. med. Wschr 83 930 1958 1958.
- 19 Alwall V Fifteen hundred treatments with the artificial kidney (dialysis, ultrafiltration) 1946—1950 Proc 1st. Int Congr Nephrol Genève/Evan, p 332 1950
- 20 Alwall V. Fluid lung in anuria-oliguria. A study of 607 cases, Proc. 8th Internat. Congr Int Med, p 10 1950
- 21 Alwall V Therapeutic and diagnostic problems in severe renal failure Scand. navian University Books Stockholm, 1953
- 22 Arthonisen P Hjeri is minutivolumen ved uremi. Nord Med 64 1033 1960
- 23 Arthonisen, P Holst E Determination of cardiac output and other hemodynamic data in uremic patients using dye dilution technique Scand J Clin. Lab Invest, 12 481 1960.
- 24 Antila V Härtel C Über die Wirkung von Palfium auf das Adrenalin Lungen-ödem der Maus Ann. Med. exp. Fenn. 37 259 1950
- 25 Auer J Gates F L Experiments on the causation and amelioration of drenaline pulmonary edema. J exp Med. 20 201 1917
- 26 Barden R Cooper D The roentgen appearance of the chest in diseases affecting the peripheral vascular system of the lungs. Radiology 51 44 1948.
- 27 Bickel M A Hl D Action comparée des différents groupes de myopathiques dans les oedemes pulmonaires expérimentaux d'origine barbiturique et adrénergique Therapi 8 801 1953
- 28 Barry K G Bennett L R M Isotonic infusion III The acute effect of the intravenous infusion of mannitol on blood and plasma volumes. New Engl. J Med. 264 1085 1961
- 29 Barry K C Mallog J P Oliguric renal failure Evaluation and therapy by the intravenous infusion of mannitol J Amer med. Ass 179 510 1962.
- 30 Baruch A. L. The use of helium in the treatment of asthma and obstructive lesions in the larynx and trachea. Am. J. Med. 9 739 1935.
- 31 Bass H E Greenberg D Singer E, Miller M A. Pulmonary changes in uremia. J Amer med. Ass. 145 74 1952.
- 32 Bass H E Singer E. Pulmonary changes in uremia. J Amer med. Ass. 144 819 1950
- 33 Battelli F Taramasi P Toxicité de la substance active des capsules surrénales. C. R. Soc. Biol. Paris 51 815 1902
- 34 Bergman F To be published 1964
- 35 Bernard C. Leçons sur la physiologie et la pathologie de système nerveux Baillière et Fils, Paris 2 344 1838.
- 36 Bernstein, L. M Blumberg B Arkis M C Osmotic diuretic treatment of refractory edema. Circulation 17 1013, 1958.
- 37 Blument L W Webster C D Elkinton, J R Acute tubular necrosis. Arch. intern. Med 104 180 1959
- 38 Borgström, K E., Ising L Lunde E Landerquist A. Experimental pulmonary edema Acta Radiologica 54 97 1960
- 39 Borgström, K E. Landerquist A Pulmonary edema. Encyclopedia of Medical Radiology Band IX. Springer Verlag, Heidelberg in press 1963.
- 40 Born G I R Acute edema in the isolated perfused lung rabbits. J Physiol. 124 502, 1954
- 41 Bouchard C Claude H Recherches expérimentales sur le drenaline C. R. Acad. Sc. 135 928, 1907.
- 42 Braun, F Experimentelles zum Lungen-ödem. Wien klin. Wschr 40 202, 1923.
- 43 Cameron C R Pulmonary oedema Brit Med J 1 965 1948.
- 44 Carr C. J Krant J C. Sugar alcohols XII The fate of poly glycol and mannitol in the animal body J Biol. Chem 121 221 1938.

- Ural dropsy. *Guy's Hosp. Rep.* (3rd series) 34 183, 1879.
102. Goodpasture E. W.: The significance of certain pulmonary lesion in relation to the etiology of influenza. *Amer J med. Sci.* 155 863, 1919.
  103. Goodrich W. A.: Pulmonary edema. A correlation of X-ray appearance and physiological changes. *Radiology* 31: 56, 1918.
  104. Gerik, T.: Postural circulatory and respiratory changes during ether and intravenous anesthesia. *Acta chir scand.* 87 Supplement 102, 1945.
  105. Gould D. M. Terrence D. J.: Pulmonary edema. *Am. J. Roentgenol.* 73 368, 1955.
  106. Greeley B. A., Eitzen J.: The pathology of rheumatic pneumonia. *Am. J. med. Sci.* 183 336 1932.
  107. Gromley C. R. Fletcher J. B. Samaha, F. A.: Cardiovascular responses in dogs to large intravenous infusions. *Canad. J. Biochem. Physiol.* 22 282, 1964.
  108. Grönlund, E. F. Grönlund, A.: Toxicity of urea and its role in the pathogenesis of uremia. *J. clin. Invest.* 33 719, 1956.
  109. Grönwall A.: Dextran and its use in colloid infusion solutions. Almqvist & Wiksell, Stockholm, Sweden, 1957.
  110. Grönwall, A. Lagergren, B.: Untersuchungen über Dextran und sein Verhalten bei perivascularer Zufuhr II. *Acta Physiol. scand.* 9 1 1945.
  111. Haddy F. J. Campbell, O. S. Vanech M. B.: Pulmonary vascular pressures in relation to edema production by airway resistance and plethors in dogs. *Amer. J. Physiol.* 161 336, 1950.
  112. Halp A.: Memoire sur les parties sensibles et irritables du corps animal. *L'annee* 1 221 1786—1790.
  113. Halpern, B. V. Cruchard S.: Prevention of the edema sign in pneumonia experimental par les antihistaminiques de synthese. *C. R. Soc Biol. Paris.* 111 1038, 1947.
  114. Halpern, B. N. Cruchard S., Vermeil G., Roux J. L.: Etude pathologique et thérapeutique de l'edème aigu du poumon experimental. *Arch. int. Pharmacodyn.* 82 423, 1950.
  115. Harrington, M. Kincaid-Smith, P. M. Macleod J.: Results of treatment in malignant hypertension. *Brit. Med. J.* 2 960 1959.
  116. Harrison, W. Liebman A. L.: The effects of increased intracranial pressure on the pulmonary circulation in relation to pulmonary edema. *Circulation* 8 834, 1953.
  117. Harrison, W. Liebman A. A.: The effects of massive infusion with special reference to pulmonary congestion and edema. *Yale J. Biol. Med.* 26 572, 1954.
  118. Heilmann, R.: La pneumopathie urémique. *Acta clin. belg.* 14 463, 1950.
  119. Henkin, R. I. Bish p. H. A.: Pulmonary clearance of radioactive iodinated serum albumin in man. *J. Lab. clin. Med.* 60 700 1962.
  120. Henkin, R. I. Marwood M. H. Murray J. F.: Uremic pneumonitis. A clinical, physiological study. *Ann. Intern. Med.* 57 1001 1962.
  121. Hennessy, P. H.: Acute pulmonary edema with special reference to experimental studies. *New Engl. J. Med.* 335 890 819 1946.
  122. Hirschfelder H. Z.: Röntgendiagnostik des Lungödem. *Fortschr. Röntgenstr.* 89 123, 1958.
  123. Hewich, G.: Die urämische Lunge. *Z. ges. inn. Med.* 3 62, 1934.
  124. Hildes, T. Krogstad, A. R. Wistrup B. F.: Pulmonary changes during the medical treatment of malignant hypertension. *Lancet* 1 800, 1959.
  125. Hodson, C. J.: Pulmonary oedema and breathing shadow. *J. Fac. Radiol. (Lond.)* 1 176, 1950.
  126. Hoppes H. C., Wistrup B. V.: Uremic pneumonitis. *Amer. Path.* 31 261 1955.
  127. Houch G. R.: Disappearance of marmoset and PAH from plasma of bilaterally nephrectomized dogs. *Amer. J. Physiol.* 163 102, 1951.
  128. Howard J. M. Frumby J. P. Ariz, P. Sala, Y.: The effect of dextran and modified gelatin in casualties with renal insufficiency. *Surg. Gynec. Obstet.* 100 207 1951.
  129. H. Lieber W. Costen, G., Harrison, T. R.: Experimental hyperosmotic heart fail-

- 74 Eaton R M. Pulmonary edema. Experimental observations on dogs following acute peripheral blood loss. *J Thorac. Surg* 16 668, 1947
- 75 Ehrlich W McIntosh J F The pathogenesis of bronchiolitis obliterans. *Arch. Path.* 13 69 1932
- 76 Etchna L W Farber S J, Berger A R Rader B Smith, W W Albert R E. Non-cardiac circulatory congestion simulating congestive heart failure. *Trans. Ass. Amer. Physcns.* 67 72, 1954
- 77 Elkinton, J R. Non utilization of mannitol in uremic patients. *Fed. Proc.* 8 41 1949
- 78 Elliot T R The action of adrenaline. *J. Physiol. Lond.* 39 401 1905
- 79 Ellis A W M. Heart failure in acute nephritis. *Quart. J. Med.* 5 533, 1938.
- 80 Emerson, H Artificial respiration in the treatment of edema of the lungs. *Arch. Intern. Med.* 3 368 1909
- 81 Eriksson P Lindholm, T Lindqvist B Sorenson, A Artificial respiration in severe renal failure with pulmonary insufficiency *Acta med. scand.* 166 81 1960
- 82 Farber S. Studies on pulmonary edema. I. The consequences of bilateral cervical vagotomy in the rabbit. *J. exp. Med.* 66 397 1937
- 83 Farber S. Studies on pulmonary edema. II The pathogenesis of neuropathic pulmonary edema. *J. exp. Med.* 66 405 1937
- 84 Farber S. Neuropathic pulmonary edema. *Arch. Path.* 30 180 1940
- 85 Fishberg A M Advances in the treatment of renal disease. *Practitioner* 175 403, 1955
- 86 Fowler N O Bloom, W L, Ward J A, Franch R H. Hemodynamic effects of hypervolemia with and without anemia. *Circulation* 14 937 1956.
- 87 Fowler N O Bloom W L, Ward J A. Hemodynamic effects of hypervolemia with and without anemia. *Circulation Res* 6 163, 1958.
- 88 Fremont-Smith, F Forbes H S. Intracocular and intra-cranial pressure. *Arch. Neurol. Psychiat.* (Chic.) 18 580 1927
- 89 Friedrichs F T. Die Brightsche Nierenkrankheit und deren Behandlung. Braunschweig 1851
- 90 Frey O Die pathologischen Lungenveränderungen nach Lähmung der Nervi vagi. Wilhelm Engelmann, Leipzig 1877
- 91 Friedberg C K Congestive heart failure of renal origin. *Amer. J. Med.* 9 164, 1950
- 92 Fritz, H Lindqvist B. Pulmonary ventilation (Becklake's index) in renal failure, with special reference to overhydrated patients with "Fluid lung" *Acta med. scand.* 169 181 1961
- 93 Funck-Brentano J L Losky-Nekhorcheff I Altman J Etude de expérimentale des manifestations cérébrales de l'intoxication pour l'eau. *Rev. franc. d'études Clin. Biol.* 4 46, 1959
- 94 Földi M Koranyi A, Szabo G Über die Entstehung anämischer Ödeme. *Acta med. scand.* 129 466 1948.
- 95 Ghalloungui P. Médecine in the days of the pharaohs. *Ciba Symp.* 9 206 1961.
- 96 Gibbon, J H Gibbon, M H Experimental pulmonary edema following lobectomy and plasma infusion. *Surgery* p. 694, 1942
- 97 Gibbon, J H Gibbon, M H Kraus C W Experimental pulmonary edema following lobectomy and blood transfusion. *J. thorac. Surg* 12 60 1942.
- 98 Gilligan R Altschule M D Volk M C The effects on the cardiovascular system of fluids administered intravenously in man. I. Studies of the amount and duration of changes in blood volume *J. clin. Invest.* 17 7 1938.
- 99 Glass A Über Beeinflussung des Adrenalins—Lungenödem durch experimentelle Verletzungen des Hirnstammes und des Sympathikus. *Arch. exp. Path. Pharmac.* 136 83, 1928.
- 100 Goldenberg M Crane R D Popper H Effect of intravenous administration of dextran a macromolecular carbohydrate in animals. *Am. J. Clin. Path.* 17 939 1947
- 101 Goodhart J F On cut dilatation of the heart as cause of death in scarla-

- Real dreyer Gay Hosp. Rep. (3rd series)  
II 153, 1879.
141. Goodpasture E. W.: The significance of certain pulmonary lesions in relation to the etiology of influenza. *Amer J med. Sci.* 153: 863, 1919
  142. Goodrich, W. A.: Pulmonary edema. A correlation of X-ray appearance and physiological changes. *Radiology* 31 58, 1948.
  143. Gorka, J.: Postural circulatory and respiratory changes during ether and intravenous asphyxia. *Acta chir. scand.* 87 Supplement 102, 1948.
  144. Gould, D. M. Terrance D. J.: Pulmonary edema. *Am. J. Roentgenol.* 33 306, 1935.
  145. Goudry B. A., Elmer, J.: The pathology of rheumatic pneumonitis. *Am. J. med. Sci.* 142, 354, 1932.
  146. Goudry C. W. Hatche J. D. Sarubaru F. A.: Cardiovascular responses in dogs to large intravenous infusions. *Canad. J. Biochem. Physiol.* 32 252, 1954.
  147. Grönlund, E. F. Grönlund, A.: Toxicity of urea and its role in the pathogenesis of uremia. *J. clin. Invest.* 38 749 1950
  148. Grönlund, A.: Dextran and its use in colloid infusion solutions. Almqvist & Wiksell, Stockholm, Sweden, 1951
  149. Grönlund, A., Ingelmann, B.: Untersuchungen über Dextran und sein Verhalten bei peritonealer Zufuhr II. *Acta Physiol. scand.* 9 1 1945
  150. Hadley F. J. Campbell O. B., Vlascher M. B.: Pulmonary vascular pressures in relation to edema production by airway resistance and plethora in dogs. *Amer J Physiol.* 161 336, 1930.
  151. Hader A.: Memoire sur les parties sensibles et irritables du corps animal. *Lancette*, I 224, 1765-1769.
  152. Halpern, B. A. Cruchard S.: Prevention de l'œdème aiguë du pommex expérimental par les inhibiteurs de la synthèse. *C. R. Soc. Biol. Paris.* 147 1938, 1947
  153. Halpern, B. N. Cruchard S. Verrell G., Amer J. L.: Etude pathologique et thérapeutique de l'œdème aiguë d pommex expérimental. *Arch. int. Pharmacodyn* 83 424, 1952.
  154. Harrington, M. Kincaid-Smith, P. McMichael J.: Results of treatment in malignant hypertension. *Brit. Med. J.* 2 909 1950
  155. Harrison, W. Liebow A. A.: The effects of increased intracranial pressure on the pulmonary circulation in relation to pulmonary edema. *Circulation* 5 821, 1952.
  156. Harrison, W. Liebow A. A.: The effects of nasal infusion with special reference to pulmonary congestion and edema. *Y. in J. Biol. Med.* 28 372, 1954.
  157. Heilmann, R.: La pneumopathie urémique. *Acta clin. belg.* 14 403 1950.
  158. Herlin, R. I. Bishop, H. A.: Pulmonary clearance of radioactive iodinated serum albumin in man. *J. Lab. clin. Med.* 60 702, 1962.
  159. Herlin, R. I. Marzetti M. H. Murray J. F.: Uremic pneumonitis. A clinical, physiological study. *Ann. Intern. Med.* 57 1001 1962.
  160. Heuserman, P. H.: Acute pulmonary edema with special reference to experimental studies. *New Engl. J. Med.* 235 590 619 1946
  161. Herrhäuser H.: Zur Röntgendiagnostik des Lungenödems. *Fortschr. Röntgenstr.* 68 124, 1956.
  162. Heuchel G.: Die urämische Lunge. *Z. ges. Inn. Med.* 2 82, 1946.
  163. Hildebrand, T. Kraggegaard A. R. Wilmstrup B.: Fatal pulmonary changes during the medical treatment of malignant hypertension. *Lancet* I 830, 1958.
  164. Hodson, C. J.: Pulmonary oedema and hazy shadow. *J. Fac. Radiol. (Lond.)* 1 178, 1950
  165. Hopp H. C., Winkler R. W.: Uremic pneumonitis. *Amer. Path.* 31 261, 1954.
  166. Houch, G. R.: Disappearance of mannitol and PAH from plasma of bilaterally nephrectomized dogs. *Amer J Physiol.* 165 102, 1951.
  167. Howard J. M. Frowby J. P. Artt, P. Sato, Y.: The fate of dextran and modified gelatine in casualties with renal insufficiency. *Surg. Gynec. Obstet.* 100 207 1961
  168. Huckabee W. Castro, G., Harrison, T. R.: Experimental hypervolemia heart fail-

- ure its bearing on certain general principles of heart failure. *Circulation* **1** 343 1950
- 130 *Iklos D* Luft R kroppens extracellulära vätskerum. *Nord Med.* **57** 13 1957
  - 131 *Ivanitskaya M A* Roentgen picture of nephrogenic edema of lungs. *Klin. Med. (Mosk.)* **21** 54 1943.
  - 132 *Jackson F* The radiology of acute pulmonary oedema. *Brit. Heart J.* **13** 503, 1951
  - 133 *Javid M* Urea—new use of an old agent. *Surg. Clin. N Amer* **907** 1958.
  - 134 *Javid M Anderson, J* The effect of urea on cerebrospinal fluid pressure in monkey before and after bilateral nephrectomy. *J. Lab. clin. Med.* **53** 484 1959
  - 135 *Javid M Settlege P* Effect of urea on cerebrospinal fluid pressure in human subjects. *J Amer med Ass.* **100** 943 1956.
  - 136 *Jensen, O L, Lindhardt, F* Röntgenförändringar ved akut lungeödem. Frem kaldt ved transfusionsbehandling. *Ugeskr Læg.* **124** 827 1962.
  - 137 *Johnson S* Experimental production and prevention of acute edema of the lungs in rabbits. *Proc. Soc. exp. Biol. (NY)* **25** 181 1927
  - 138 *Josze O Bloch, L.* Oedème aigu du poumon et périmérial par injection intraveineuse d'extraits d'écorce corticale de capsules surrénales. *Bull. Soc. Méd. Paris.* **96** 55 1908.
  - 139 *Jung H* Zucht und Haltung der wichtigsten Laboratoriumsversuchstiere VEB Gustav Fischer Verlag Jena 1958
  - 140 *Kabanchik M Aletmans V* Pulmonary uremia. *Pres. méd. argent.* **38** 2143 1951
  - 141 *Kasanen, I Lahesmaa R* The effect of blood transfusion and fluid therapy on the vital capacity and blood circulation in the uremic patients. *Acta med scand* **172** 555 1963
  - 142 *Kassirer J P Schwartz W B* Acute glomerulonephritis. *Nw Engl. J. Med.* **265** 686, 1961
  - 143 *Kerääläinen, T O Passonen, M K* Pulmonary edema produced by cervical vagotomy. *Ann. Med. exp. Penna* **36** 87 1958.
  - 144 *Khomlakos I C Kainosa S S* X-ray diagnosis of nephrogenic pulmonary edema. *Urologia* **26** 17 1961
  - 145 *Klima, R Rosegger H* Eigenartige Krankheitsbilder zufolge von Lungenödem bei Niereninsuffizienz. *Med. Klin.* **32** 85, 1936.
  - 146 *Knoll P.* Bemerkungen zur Infusion blutwarmer physiologischer Kochsalzlösung in das Gefäßsystem. *Arch. exp. Path. Pharmac.* **36** 263 1925
  - 147 *Koenig H Koenig R* Studies on the pathogenesis of ammonium pulmonary edema. *Amer J Physiol.* **158** 1 1949
  - 148 *Köhler D Barb M* Variation des liquides du poumon dans l'œdème pulmonaire aigu d'origine barbiturique Comparaison avec l'œdème adrénalique. *C. R. Biol. (Paris)* **148** 290 1954
  - 149 *Korner P L.* The effect of a renaline-induced systemic vasoconstriction on the formation of pulmonary edema. *Aust. J. exp. Biol. med. Sci.* **31** 405, 1953
  - 150 *Kraus F* Über Lungenödem. *Z. exp. Path. Ther.* **14** 407 1913
  - 151 *Krambhan E B Chanalin L.* Studies in experimental plethora in dogs and rabbits. *J exp Med.* **35** 847 1922.
  - 152 *Lagrang E P met R* Recherches expérimentales sur l'œdème pulmonaire. *Arch. Int. Pharmacodyn.* **9** 445 1949
  - 153 *Land A M Gutting R A Larson P S.* The size of the extracellular fluid compartment before and after massive infusion. *Amer J Physiol.* **130** 421 1940
  - 154 *Landsteiner K* Über Agglutinationserscheinungen normal menschlichen Blutes. *Wien. klin. Wochr.* **14** 1152, 1901
  - 155 *Langendorf R Peck A* Elektrokardiogramm bei akuter Nephritis. *Acta med. scand* **91** 1 1938.
  - 156 *Last J M M D nald G O Jane R A Bond E. E.* Rate of equilibration of fluid and man between plasma and interstitial water in edematous states. *J. Lab. clin. Med.* **39** 63 1953.
  - 157 *Lazarus J A, Srebro skaya, I A* The

- pathogenesis of pulmonary edema. *Sovets. Med* 24 87 1960
- 165 *Lepoint M* Experiences sur le principe de la le p 212 D'Hautel, Paris 1812.
  - 159 *Leydne* Quoted from Luskada, 1940
  - 160 *Lefang, M* Bernard J L'image radiologique d'un cas d'œdème aigu du poumon d'origine rénale chez un enf n1 Soc. Med 116p. Paris 177 183 1937
  - 161 *Lepeschkin, E.* Das Elektrokardiogram Verlag Theodor Steinkopff Dresden, 1937
  - 162 *Levine H D* Spontaneous changes in the normal rabbit electrocardiogram *Amer Heart J* 24 200 1942.
  - 163 *Lezau P* *Stigand H* Glomerulonephritis with bilateral lung purpura. *Act med. scand.* 163 403, 1960.
  - 164 *Lorber F* Lung edema following bilateral vagotomy *J asp Med* 70 117 1939
  - 165 *Lower R.* *Tractat de Corde.* London, 1669. (Quoted from Short 1914)
  - 166 *Lower R.* *Philosoph Trans. Royal Soc. London.* 1 128 1667—1668 (Quoted from Short 1914)
  - 167 *Luskada A.* Beitrag zur Pathogenese und Therapie des Lungödems und des Asthma cardiacum *Arch exp Path. Pharmac.* 123 212, 1928
  - 168 *Luskada, A.* The pathogenesis of paroxysmal pulmonary edema *Medicine* 19 478, 1940.
  - 169 *Luskada, A.* Therapy of paroxysmal pulmonary edema by antifoaming agents *Circulation* 2 872, 1950
  - 170 *Luskada A.* *Sarnoff S J* Paroxysmal pulmonary edema consequent to stimulation of cardiovascular receptors I II III *Am Heart J* 81 270, 1946
  - 171 *Lundquist I* (in collaboration with A. Åhrwall, A. Tornberg.) On the artificial kidney XVI The efficacy of the dialyser—ultrafilter intended for human use, including preliminary report on treatment of edematous patient by means of ultrafiltration *Acta med. scand* 143 307 1952
  - 172 *Lundquist I* *Komstgord* para. VIII. Ultrafiltrationsbehandling i11 experimen till rikets h111111 Nord Med 50 1259 1953
  - 173 *Luskada M* Über die Entstehung des Lungödems. *Bell' path. Anat.* 13 401 1803.
  - 174 *MacKee, I H* Symposium on oedema. III. Review of the patho-physiology of pulmonary oedema. *N. S. med. Bull.* 29-337, 1908.
  - 175 *Macht D L., Ting G C.* The effect of some polyhydric alcohol on the behavior of rats in the circular maze *Amer Physiol.* 60 496, 1922.
  - 176 *Madeit* Quoted from Luskada 1940.
  - 177 *Manery J F* *Hastings A. B* The distribution of electrolytes in mammalian tissues. *J Biol. Chem.* 127 657 1920
  - 178 *Marx G. F* *Ketning J W H Lin, L. R.* Diffusional hyperkalemia during transurethral resection of the prostate. *J Amer med. Ass.* 174 1834 1960.
  - 179 *Marx G. F* *Rothman R., Olin, L. R.* Intravenous infusions of 10 per cent cytol, 8 per cent dextrose/water or dextrose in dogs. *J Urol (Baltimore)* 84 424, 1960
  - 180 *Marrison, S* *Petterson, R. M* *Mace I.* *Le Brie S. J* *Mayerman, P* Permeability of lymphatic vessels *Amer J Physiol.* 203 98, 1962.
  - 181 *Mendshell R. M* *Brumaine P M* *Gertel B W* ter sodium and potassium content of human, guinea pig and rabbit lung *Proc. Soc exp Biol.* 22 318, 1923.
  - 182 *M Lien, P* Oedema pulmonaire et uremie C. R. XIII. Congr 1 internat. Med. Paris 4 219, 1900.
  - 183 *Merrill J P* *Smith S., Callahan, E. F* *Thoma, G U* The use of an artificial kidney II. Clinical experiences *J clin. Investig.* 29 423, 1950
  - 184 *Miller J* *Matthews, S. A.* A study of the mechanical factors in experimental acute pulmonary edema *Intern. Int Med J.* 236 1909
  - 185 *Moll, A., H L.* Blood transfusion in clinical medicine Blackwell Scientific Publications, Oxford 1961
  - 186 *Moore, I H* *Morgan, D R.* Experimental pulmonary edema. *Arch. Path* 21 563, 1926
  - 187 *Monte, Y* Hemodynamic and lectrolyte studies in acute glomerulonephritis. *Ciba. Res. Proc* 3 206, 1957



- ure its bearing on certain general principles of heart failure *Circulation* 1 343 1950
- 130 *Illoa D Luft R* Kroppens extracellulära vätskerum. *Nord Med* 57 137 1957
- 131 *Isandtskagn M A* Roentgen picture of nephrogenic edema of lungs. *Klin. Med (Mosk.)* 21 54 1943.
- 132 *Jackson F* The radiology of acute pulmonary oedema. *Brit. Heart J* 13 603, 1951
- 133 *Javid V* Urea—new use of an old agent. *Surg. Clin. N Amer* 907 1958.
- 134 *Javid M Anderson J* The effect of urea on cerebrospinal fluid pressure in monkey before and after bilateral nephrectomy. *J. Lab. clin. Med.* 53 484 1959
- 135 *Javid M Selllage P* Effect of urea on cerebrospinal fluid pressure in human subjects. *J Amer med. Ass.* 160 913, 1956.
- 136 *Jepsen O L Lindhardtien F* Röntgenforandringer ved akut lungeødem. *Frem Lægt ved transfusionsbehandling Ugeskr Læg.* 124 827 1902.
- 137 *Jinason S* Experimental production and prevention of acute edema of the lungs in rabbits. *Proc. Soc. exp. Biol. (NY)* 25 181 1927
- 138 *Josue O Bloch L* Oedème aigu du poumon expérimental par injection intra osseuse d'extraits de couche corticale de capsules surrénales. *B. II. Soc. Méd. Paris.* 28 65 1908.
- 139 *Jung S* Zucht und Haltung der wichtigsten Laboratorien ersuchsre VEB G. I. Fischer Verlag Jena. 1958.
- 140 *Kabanchik V Kleimans M* Pulmon uremico. *Pres. méd. argent.* 38 2143, 1951
- 141 *Karsten A Lohman R* The effect of blood transfusion and fluid therapy on the vital capacity and blood circulation in uremic patients. *Acta med. scand.* 172 553, 1962
- 142 *Kassirer J P Schwartz B B* Acute glomerulonephritis. *New Engl J Med* 265 683, 1961
- 143 *Kegrellinen T O Poosonen, M K.* Pulmonary edema produced by cervical vagotomy. *Ann. Med. exp. Penn.* 36 87 1958.
- 144 *Kh mlakov I C Kalnosa S S* X-ray diagnosis of nephrogenic pulmonary edema. *Urologia* 26 17 1961
- 145 *Kilma R Rosegger H* Eigenartige Krankheitsbilder auf ige von Lungenödem bei Nierenluxuffizienz. *Med. Klin.* 52 85, 1956.
- 146 *Knoll P.* Bemerkungen zur Infusion blut warmer physiologischer hochkonzentration in das Gefäßsystem. *Arch. exp. Path. Pharmac.* 30 203 1905
- 147 *Koenig H Koenig R* Studies on the pathogenesis of ammonium pulmonary edema. *Amer J Physiol.* 158 1 1949
- 148 *K öler D., Barbe M.* Variation des liquides d poumon dans l'oedème pulmonaire aigu d'origine barbiturique Comparaison avec l'oedème adrénalique. *C. R. Biol. (Paris)* 148 290 1954
- 149 *Korner P L.* The effect of noradrenaline—induced systemic vasoconstriction on the formation of pulmonary edema. *Aust. J exp Biol. med. Sci* 31 403, 1953.
- 150 *Kraus F* Über Lungenödem. *Z. exp. Path. The* 14 402, 1913.
- 151 *Kumbha E. B Channin L.* Studies on experimental plethora in dogs and rabbits. *J exp. Med.* 35 817 1922.
- 152 *Lagrange E. Pomet R* Recherches expérimentales sur l'oedème pulmonaire. *Arch. Int Pharmacodyn.* 79 445 1949
- 153 *Lands A M Gutting R A Larson P S* The size of the extracellular fluid compartment before and after massive infusions. *Amer J Physiol.* 130 421 1946.
- 154 *Landst inner K* Über Agglutinationserscheinungen normalen und nephrotischen Blutes. *Wien. Klin. Wochr* 14 1132 1901
- 155 *Langendorf R Pick A* Elektrolytogramm bei akuter nephritis. *Acta med. scand.* 91 1 1938
- 156 *Lat J M M Donald C G* *J new R A B and E C* Rate of equilibration of fluid and man fluid between plasma and interstitial water in edematous states. *J Lab. Clin. Med.* 39 62, 1952.
- 157 *Lauri J L, S. Brown J G* *J L.* The

- pathogenesis of pulmonary edema. *Sovets. Med.* 21 III 1960
164. *Leydolt M* Experiences sur la principe de la le p 212 D'Hautel, Paris 1812
165. *Leydolt M* Quoted from Laisada, 1910.
166. *Lefang M* Bernard J L'image radiologique d'un cas d'œdème aigu du poumon d'origine rénale chez un enfant. *Soc. Med. Hôp. Paris* 177 183, 1937
167. *Lepeschitz E.* Das Elektrokardiogramm. Verlag Theodor Stinkoff Dresden, 1937
168. *Leslie H B* Spontaneous changes in the normal rabbit electrocardiogram. *Amer Heart J* 28 209 1942.
169. *Lessee P* Sigurd H Glomerulonephritis with initial long-perpura. *Acta med. scand.* 163 403 1960.
170. *Leslie V* Lung edema following bilateral apotomy. *J. exp. Med.* 70 117 1939.
171. *Lowry R* Tractatus de Corda. London, 1680 (Quoted from Short 1944)
172. *Lowry R* Philosoph. Tra. a. Royal Soc. London, 1 128 1665—1668. (Quoted from Short 1944)
173. *Laisada, A* Beitrag zur Pathogenese und Therapie des Lungödema und des Asthma cardiale. *Arch. exp. Path. Pharmacol.* 132 213, 1922.
174. *Laisada A* The pathogenesis of paroxysmal pulmonary edema. *Medicine* 29 475, 1940.
175. *Laisada, A.* Therapy of paroxysmal pulmonary edema by sulfonamide agent. *Circulation* 2 872, 1950
176. *Laisada, A* Sarnoff S J Paroxysmal pulmonary edema consequent to stimulation of cardiovascular receptors I II. *III. Am. Heart J* 31 370 1946
177. *Linderquist J* (I collaboration with X. Alwall, A Tornberg) On the artificial Kidney. XXI The efficacy of the dialyser—ultrafilter intended for human use including preliminary report on the treatment of oedematous patient by means of ultrafiltration. *Acta med. scand* 143 307 1952.
178. *Linderquist J* Eon igjord jure. VIII Christlirationsbehandling af peritonenalt Jiskelings hos kaakin. *Nord Med* 50 1229 1953.
179. *Léauté M* Über die Entstehung des Lungödems. *Beitr. path. Anat* 14 401 1893.
180. *MacKee, A H* Symposium on oedema. III Review of the patho-physiology of pulmonary oedema. *N. S. med. Bull.* 39 332, 1960.
181. *Macht D L, Ting G. C.* The effect of some polyhydric alcohol on the behavior of rats in the circula maze. *Amer Physiol.* 60 406, 1922.
182. *Maloff* Quoted from Laisada 1940
183. *Mavery J F* Hastings, A. H The distribution of electrolytes in mammalian tissues. *J Biol. Chem.* 177 637 1939
184. *Marx, G. F* Keenring J W O Lin, L. R Dilational hypovolemia during transurethral resection of the prostate. *J Amer med. Ass.* 174 1834 1960
185. *Marx, G. F* Rathens, R O Lin, L. R Intravenous infusions of 10 per cent cytol, 5 per cent dextrose/water or dextran in dogs. *J Urol (Baltimore)* 81 424 1960
186. *Margerson, S* Patterson, R. M M Kee A Le Bre S J Margerson, P Permeability of lymphatic vessels. *Amer J Physiol.* 201 99, 1962
187. *Merrill R M* Rasmussen P M Gerol B W ter sodium and potassium content of human, guinea pig and rabbit lung. *Proc Soc. exp Biol.* 43 318, 1943
188. *Merklen, P* Oedema pulmonaire et urémique. *C. R. XIII Congr. Internat. Med.* Paris 4 219 1900
189. *Merrill J P* Smith, S., Gifford E. F Thora, G. C The use of an artificial kidney II Clinical experiences. *J clin. Invest.* 29 323, 1950
190. *Mull J* Matthew S. A A study of the mechanical factors in experimental acute pulmonary edema. *Intern. J. Med.* 4 330, 1960
191. *Mathison, P L* Bloodless anatomy of renal medicine. Blackwell Scientific Publications, Oxford 1961
192. *Moss, J H* Morgan, D R Experimental pulmonary edema. *Arch. Path.* 21 45 1936
193. *Moss J* Hemodynamic and electrolyte studies in acute glomerulonephritis. *Clin. Res. Proc* 3 206, 1957

- 187 Morrison, A E., Lundg J S Essex H E An evolution of replacement fluids in laboratory animals following control hemorrhage. *Circulation* 5 208, 1952.
- 188 Valrow P J., Oestreich H M Swan, R C Measurement of extracellular fluid volume of nephrectomized dogs with mannitol, sucrose, thiosulfate and radiolabeled Amer J Physiol 185 170 1956.
- 189 Murphy F D Correl H Grill J C.: The effects of intravenous solutions on patients with and without cardiovascular defects. *J Amer med. Ass.* 116 104 1941
- 190 Müller W.: Die Abhängigkeit des arteriellen Druckes von der Blutmenge. Arbeiten aus der physiol. Anstalt zu Leipzig, p. 150 1874
- 191 Möller H Physiologie und Klinik der Bluttransfusion. Gustav Fischer Verlag, Jena 1960
- 192 Nairn R C.: Oedema and capillary anoxia. *J Path.* 63 213, 1951
- 193 Nemir R L. Bernbaum, S L.: Pulmonary edema occurring during the course of renal azotemia. *J Dis. Child.* 95 516 1958.
- 194 Nessa C B Ripler L G The roentgenological manifestations of pulmonary edema *Radiology* 37 35 1941
- 195 Nickel J F Lawrence J Leljer D B Bradley S E Renal function electrolyte excretion and body fluids in patients with chronic renal insufficiency before and after sodium deprivation. *J clin. Invest.* 32 68, 1953.
- 196 Nieth H Bedrohliche Situation bei akuter Glomerulonephritis. *Internist* 2 45 1961
- 197 Olsson O Some radiological problems connected with Bright's disease *Brit. J Radiol.* 27 83, 1954
- 198 Olsson O Roentgen examination of the kidney and the ureter *Encyclopedia of urology* vol 5, edited by Alken et al. Springer Verlag, Berlin 1962.
- 199 Opdyke D F Duomarcio J Dillon, W H Schreiber H Little R C Seely R D Study of simultaneous right and left atrial pressure pulses under normal and experimentally altered conditions. *Amer J Physiol.* 154 258, 1958.
- 200 Paasonen M K Effect of some antihistaminics on adrenalinic responses in animal tests. *Ann. Med. exp. Fran.* 31 suppl. 7 1953
- 201 Pace A Kline L., Scochman, H K Harjesit M Studies on body composition. IV Use of radioactive hydrogen for measurement in vivo of total body water *J Biol. Chem.* 168 459 1947
- 202 Paine R Butcher H R Howard F A., Smith A B Smith, J R Observations on mechanisms of edema formation in the lungs. *J Lab. clin. Med.* 34 1344 1949
- 203 Painter E. E Total body water in the dog *Amer J Physiol.* 129 744 1940.
- 204 Pattle R. E. Failure of local anoxia in rabbit lung to produce or intensify pulmonary oedema. *J Path. Bact.* 72 211 1956.
- 205 Perret L., Kuhlbeck B Lungförändringar vid akut nefrit. *Finska Läk-Sällsk. Handl.* 99 187 1956.
- 206 Poulsen, T.: Quantitative estimation of pulmonary oedema in mice *Acta pharmacol. (kbb)* 10 117 1954.
- 207 Poulsen, T.: Investigations into the pulmonary oedema produced in mice by carbon dioxide *Acta pharmacol. (kbb)* 10 201 1954
- 208 Poulsen T.: Experimental investigations into the effect of morphine and ally propyl on carbon-dioxide provoked pulmonary oedema in mice. *Acta pharmacol. (kbb)* 10 246, 1954
- 209 Poulsen, T.: Experimental investigations into the effect of ether on carbon-dioxide provoked pulmonary oedema in mice. *Acta pharmacol. (kbb)* 10 253, 1954
- 210 Poulsen T.: Experimental investigations into the effect of atropine, sympatholytics, sodium nitrate and promethazine on pulmonary oedema provoked by carbon-dioxide. *Acta pharmacol. (kbb)* 10 379 1954
- 211 Poulsen T Kuldioxidets anaesteterende och lungedemframkallande virkning. Copenhagen 1958.
- 212 Prichard M M L., Daniel P M Ardran G M Peripheral ischaemia of the lung *Brit. J Radiol.* 27: 93 1954
- 213 Randall R. E., Glacier J S Liggett M.

Nephritis with lung haemorrhage. *Lancet* 1 490, 1943.

215. *Asperger F* Rapid microchemical methods for blood and CSF examinations. Grunewald Station, Inc. New York 1942.
216. *Reichman, F* Studies on the pathogenesis of pulmonary edema following bilateral vagotomy. *Ann. Intern. Med.* 31 590, 1948.
217. *Remick, R. A., Leng A. H., Cope A. M.* Pulmonary manifestations of aortemia. *Am. J. Roentgenol.* 45 802, 1941.
218. *Rennex, S.* On the roentgenological picture of pulmonary edema. *Acta radiol. (Stockh.)* 30 109 1948.
219. *Häfer G.* Die Therapie des Lungenödems. *Internist (Berl.)* 1 79 1960.
220. *Kiercher W.* Beitrag zum Cardial-1 Lungenödem. *Arch. exp. Path. Pharmacol.* 197 839 1941.
221. *Roberts J. O.* Urea as an agent for the reduction of intra-cranial pressure. (Quoted from Javid & Seilinger 1950.)
222. *Kells, J. O.* Urea is not equally distributed between the water of the blood cells and that of the plasma. *J. Biol. Chem.* 151 829 1943.
223. *Rekha, E.* II perimenteller Beitrag zur Pathologie und Therapie der Spätfolgen der durch Phosgen erzeugten Lungenödems. *Schweiz. med. Wochschr.* 70 641, 1948.
224. *Roubier C., Planchon M.* Sur certains aspects radiographiques de l'œdème pulmonaire chez les cardiaques aortémiques. *Arch. med. chir. Appar. resp.* 3 189 1934.
225. *Schiavo S., D'Onofrio T.* Ricerche sull'edema pericardiale. *Sperimentale* 107 433, 1957.
226. *Shaffer E. S.* Experiments on the cervical vagus and sympathetic. *Quart. J. exp. Physiol.* 12 231, 1920.
227. *Shiff M.* Gesamtheitliche Beiträge zur Physiologie. I. p. 330—410. B. Benda Lausanne, 1901.
228. *Schwartz, I. L., Bradd E. S., Maxwell M. H.* Comparison of the clearance of distribution, renal and extrarenal clearances of insulin and mannitol in man. *J. clin. Invest.* 29 517 1950.
229. *Se brownage, I. A.* The study of protein metabolism of the transudate in some types of experimental pulmonary edema. *Pat. Fisiol. exp. Ter.* 4 80, 1960.
230. *Shaffer E. S.* Experiments on the cervical vagus and sympathetic. *Quart. J. exp. Physiol.* 12 231, 1920.
231. *Sharpey-Shafer E. P.* Transfusion and the anemic heart. *Lancet* 2 290, 1948.
232. *Sharpey-Shafer E. P., Wallace J.* Circulatory overloading following rapid intravenous injections. *Brit. Med. J.* 2 304, 1942.
233. *Skert R. H. H.* Pulmonary changes in rabbits produced by bilateral vagotomy. *J. Path.* 55 335, 1944.
234. *Sjöstrand T.* On the principles for the distribution of the blood in the peripheral vascular system. *Skand. Arch. Physiol.* 71 suppl 5 1935.
235. *Smith H. W.* Principles of renal physiology. Oxford University Press, Inc. New York 1946.
236. *Smith, W. H., Flakstein, N., Smith, H. W.* Renal excretion of bacillos (sorbitol, mannitol and dulcitol) and their derivatives (sorbitan, isomannide and sorbide) and of endogenous creatinine-like chromogen in dog and man. *J. Biol. Chem.* 125 221 1940.
237. *Steffensen, E. A.* Some determination of the total body water in man by means of intravenous injections of urea. *Acta physiol. scand.* 13 282, 1947.
238. *Steiche M. H.* Experimental uremick-renal enteritis. *Arch. Intern. Med.* 42 835, 1928.
239. *Stone C. A., Loebe E. R.* Effect of various drugs on epinephrine induced pulmonary edema in rabbits. *Proc. Soc. exp. Biol. (N. Y.)* 71 122, 1949.
240. *Tessier J.* De l'œdème aigu du poumon. C. R. XIII Congr. Internat. Med. Paris 4 190 Marseon, Paris 1900.
241. *Fähler, H.* Urtümliche Flüssigkeitsmenge und hypertens. Nephropathie. *Wochschr. Akad. med. Wiss.* 15 390, 1929—30.

- 187 Morrison, A. E., Landy J S Essex H E An evolution of replacement fluids in laboratory animals following control hemorrhage *Circulation* 5 203, 1952.
188. Malrow P J Oestreich H M Swan, R C Measurement of extracellular fluid volume of nephrectomized dogs with mannitol, sucrose thiosulfate and radioisotope *Amer J Physiol.* 185 179 1966.
- 189 Varugh F D Correl H., Grill J C: The effects of intravenous solutions on patients with and without cardiovascular defects. *J Amer med. Ass.* 116: 104 1941
- 190 Müller W Die Abhängigkeit des arteriellen Druckes von der Blutmenge. *Arbeiten aus der physiol Anatalt zu Leipzig* p. 169 1874
- 191 Möller H.: Physiologie und Klinik der Bluttransfusion Gustav Fischer Verlag, Jena 1960
192. Aalén R C.: Oedema and capillary anoxia. *J Path.* 63 213, 1951
193. Nemir R L. Bernbaum S L.: Pulmonary edema occurring during the course of renal azotemia *J. Dia. Chlid.* 95 516, 1958.
- 194 Vessa C B Rigler L G The roentgenological manifestations of pulmonary edema. *Radiology* 37 33, 1941
- 195 Nickel J F Lawrence V Leifer D B Bradley S E Renal function electrolyte excretion and body fluids in patients with chronic renal insufficiency before and after sodium deprivation. *J clin. Invest.* 32 68, 1953
- 196 Nieh H Bedröhlche Situation bei akuter Glomerulonephritis. *Internist* 2 45, 1961
- 197 Olsson O Some radiological problems connected with Bright's disease. *Brit J Radiol.* 27 86 1954
198. Olsson O Roentgen examination of the kidney and the ureter *Encyclopedia of urology* vol. 5, edited by Alken et al. Springer Verlag Berlin 1962.
- 199 Opdyke D F Du marco J Dillon, W H Schreiber H Little R C Seely R D Study of simultaneous right and left atrial pressure pulses under normal and experimentally altered conditions. *Amer J Physiol.* 154 258, 1948
200. Passonen, M A Effect of some animal histamines on adrenaline responses in animal tests. *Ann. Med. exp. Fenn.* 31 suppl. 7 1953
201. Pace N Kline L., Seuchman, H K Harfenist M Studies on body composition. IV Use of radioactive hydrogen for measurement in vivo of total body water *J Biol. Chem.* 168 459 1947
202. Paine R Butcher H R Howard F A., Smith A. B Smith, J R Observations on mechanisms of edema formation in the lungs. *J Lab. clin. Med.* 34 1544 1919
203. Paintner E. E. Total body water in the dog *Amer J Physiol.* 129 744 1940.
- 204 Pottle R. E. Failure of local anoxia in rabbit lung to produce or intensify pulmonary oedema. *J Path. Bact.* 72 211 1956.
205. Perret L., Kuhlböck B Lungförödning vid akut nefrit. *Finska Läk. Sällsk. Handl* 99 187 1936.
206. Poulsen T Quantitative estimation of pulmonary oedema in mice *Acta pharmacol (kbb)* 10 117 1954
- 207 Poulsen, T Investigations into the pulmonary oedema produced in mice by carbon dioxide *Acta pharmacol. (kbb)* 10 201 1954
208. Poulsen T Experimental investigations into the effect of morphine and ally propymal on carbon-dioxide provoked pulmonary oedema in mice. *Acta pharmacol. (kbb)* 10 246, 1954
- 209 Poulsen T Experimental investigations into the effect of ether on carbon-dioxide provoked pulmonary oedema in mice. *Acta pharmacol. (kbb)* 10 253, 1954.
- 210 Poulsen, T Experimental investigations into the effect of atropine sympatholytics, sodium nitrate and promethazine on pulmonary oedema provoked by carbon-dioxide *Acta pharmacol. (kbb)* 10 370 1954
- 211 Poulsen T Kuldioxidets anaesthetiserende och lungeødemframkaldende virkning. Copenhagen 1956.
- 212 Prichard M M L., Daniel P M Ardran, G M Peripheral ischaemia of the lung *Brit. J. Radiol.* 27 83 1954
213. Randall R E Glatzer J S Liggett M

- Nephritis with lung haemorrhage *Lancet* 1 499, 1952.
- 213 a. *Happaport F.* Rapid microchemical methods for blood and CSF examinations. Grune and Stratton, Inc. New York 1953.
214. *Richman, F.* Studies on the pathogenesis of pulmonary edema following bilateral vagotomy *Am. Heart J* 31 890, 1946.
215. *Reidich, R. A., Long, J. H. Cove, A. M.* Pulmonary manifestations of azotemia. *Am. J. Roentgenol.* 46 802, 1941.
216. *Brunner S.* On the roentgenological picture of pulmonary edema. *Acta radiol. (Stockh.)* 30 160 1944.
217. *Reiter G.* Die Therapie des Lungenödems. *Internist (Berl.)* 1 29, 1950.
218. *Meckert W.* Beitrag zum Cardiorespirationsödem. *Arch. exp. Path. Pharmac.* 197 828, 1941.
219. *Robert J. O.* Urea as an agent for the reduction of intra-cranial pressure. (Quoted from Javid & Settlage 1956.)
220. *Redz J. O.* Urea is not equally distributed between the water of the blood cells and that of the plasma *J. Biol. Chem.* 151 579 1943.
221. *Reiffers, E.* Experimenteller Beitrag zu Pathologie und Therapie der Spätfolgen der durch Phosgen erzeugten Lungenödems Schweiz. med. Wochschr. 70-641 1940
222. *Reubier C., Planchon, M.* Sur certains aspects radiographiques de l'œdème pulmonaire chez les cardio-rouseux azotémiques. *Arch. med. chir. Appar. resp.* 2 180 1934.
223. *Schone S., D'Osofi T.* Ricerche sull'uremia sperimentale. *Sperimentale* 107 435, 1937
224. *Shaffer E. S.* Experiments on the cervicalagus and sympathetic. *Quart. J. exp. Physiol.* 12 231 1920.
225. *Shuff M.* Gesammelte Beiträge zur Physiologie. I. p. 329-410. B. Benda Lausanne 1884.
226. *Schwartz, I. L., Brodd E. S. Maxwell, M. H.* Comparison of the volume of distribution, renal and extrarenal clearances of inulin and mannitol in man. *J. clin. Invest.* 29 517 1950
227. *Serebrowsky, I. L.* The study of protein metabolism of the tracheate in some types of experimental pulmonary edema. *Pat. Fiziol. exp. Ter* 4 60 1900.
228. *Shaf E. S.* Experiment on the cervicalagus and sympathetic. *Quart. J. exp. Physiol.* 12 231 1920.
229. *Sharpey-Shaffer E. P.* Transfusion and the anemic heart. *Lancet* 2 296, 1913.
230. *Sharpey-Shaffer E. P. Wether J.* Circulatory overloading following rapid intravenous injections. *Brit. Med. J* 2 301 1942.
231. *Short, R. H. D.* Pulmonary changes in rabbits produced by bilateral vagotomy *J. Path.* 56 353, 1944.
232. *Sjöstrand T.* On the principles for the distribution of the blood in the peripheral vascular system. *Scand. Arch. Physiol.* 71 suppl. 5, 1935.
233. *Smith, H. W.* Principles of renal physiology Oxford University Press, Inc. New York 1956.
234. *Smith, W. W. Flatau, N. Smith, H. W.* Renal excretion of hexitol (sorbitol, mannitol and dulcitol) and their derivatives (sorbitan, homomide and sorbide) and of endogenous creatinine-like chromogen in dog and man. *J. Biol. Chem.* 125 221 1940.
235. *Steffensen, K. A.* Some determination of the total body water in man by means of intravenous injections of urea. *Acta physiol. scand.* 13 262, 1947
236. *Stetcher M. H.* Experimental uremic enteritis. *Arch. intern. Med.* 42, 835, 1928.
237. *Stone C. A., Lewis E. R.* Effect of various drugs on epinephrine induced pulmonary edema in rabbits. *Proc. Soc. exp. Biol. (N. Y.)* 71 122, 1942.
238. *Teissier J.* De l'œdème aigu du poumon. C. R. XIII Congr. Internat. Med. Paris 4 190, M. 1900, Paris 1900.
239. *Tölgner, H. U.* Inakische Flüssigkeitslunge und hypertensive Encephalopathie. *Bull. Schweiz. Akad. med. Wiss.* 15 206 1939

- 240 Traube L. *Gesammelte Beiträge zur Pathologie und Physiologie*. I p 1—135, August Hirschwald, Berlin 1871
- 240 a. *Tybjerg Hansen A* Osmotic pressure effect of the red blood cells—possible physiological significance *Nature* 190 504 1961
- 241 *Udvardy L.* Form und Grössenveränderungen des Herzens bei Nierenkrankungen *Fortschr Röntgenstr* 54 559 1930.
- 242 *Valantia A M I* Opera, Venice, Epistola VIII art. 30 1740 (Quoted from Legallois 1812.)
- 243 *Vanpeperstraete F.* Effect de la trachéotomie et de l'aspiration bronchique sur l'œdème pulmonaire aigu adrénalinique du lapin. *C. R. Soc. Biol. (Paris)* 131 2 1957
- 244 *Vanquellin & Segales* Quoted from Strelcher 1928.
- 245 *Vieussens R.* *Traité du Cœur* p 132, Toulouse 1715 (Quoted from Legallois.)
- 246 *Visscher M B Haddy F J Stephens G* The physiology and pharmacology of lung edema. *Pharmacol. Rev.* 8 389 1956.
- 247 *Warthen, H J* Massive intravenous injections. *Arch. Surg.* 30 109 1935
- 248 *Wasserman A Loeb L, Mayerson, H S* Capillary permeability to macromolecules. *Circulat. Res.* 3 504 1955.
- 249 *Weed L H McAtibben, P S* Pressure changes in the cerebro-spinal fluid following intravenous injection of solutions of various concentrations. *Amer J Physiol.* 48 512, 1919
- 250 *Welch W H* Zur Pathologie des Lungenödems. *Virchows Arch. path.* *Amer* 72 375, 1878.
- 251 *Werkenthin M* The roentgenological aspect of lung edema *Am. J Roentgen* 1. 41 183 1930
- 252 *Westermark Y* Roentgen studies of the lungs and heart. The University of Minnesota Press Minneapolis, 1943.
- 253 *Whitehall M R Longcope W T Williams R* The occurrence and significance of myocardial failure in acute hemorrhagic nephritis. *Bull. Johns Hopk. Hosp* 63 83 1939
- 254 *Wiggers C J.* Physiology in health and disease p. 695, Lea & Febiger Philadelphia 1939
- 255 *Wills n J G* Pulmonary oedema in acute glomerulonephritis. *Arch. Dis. Child.* 36 681 1961
- 256 *Wood E H Moe G A* The measurement of edema in the heart lung preparation *Amer J Physiol.* 136 500 1942.
- 257 *Yeomans A. Porte R R, Swank R L.* Observations on certain manifestations of circulatory congestion produced in dogs by rapid infusion. *J. clin. Invest.* 22 33, 1943.
- 258 *Young I E Pearce J E Steven n J A.* Renal responses to hypertolemia in the dog *Canad. J Biochem.* 33 800 1955
- 259 *Zaldívar R. A C Farinas L.* Pulmonarotemico o edema subagudo del pulmón en los renales. *Rev. Cuba Cardiol* 14 141 1954.
- 260 *Zdenky E.* Beiträge zur Kenntnis der kardialen Lungenstauung auf Grund röntgenologischer klinischer und anatomischer Untersuchungen. *Wiener Arch. inn. Med.* 18 461 1920
- 261 *Zdonsky E.* Über das Röntgenbild des Lungenödems, gleichzeitig ein Beitrag zur Frage der Pathogenese des Lungenödems. *Röntgenpraxis*, 5 248, 1953.
- 262 *Zettergren, L.* Uremic Lung *Acta Soc. Med. upsallen* 60 161 1955.





- 240 Traube L. Gesammelte Beiträge zur Pathologie und Physiologie I. p 1—135, August Hirschwald, Berlin 1871
- 241 Tybjaerg Hansen, A. Osmotic pressure effect of the red blood cells—possible physiological significance *Nature* 190 504 1961
- 242 Udoardy L. Form und Grössenveränderungen des Herzens bei Nierenkrankungen. Fortschr Röntgenr. 34 539 1936.
- 243 Valsalva A. M. V. Opera, Venice, Epistola XIII art. 30 1740 (Quoted from Legallois 1812)
- 244 Vanpeperstraete F. Effect de la trachéotomie et de l'inspiration bronchique sur l'œdème pulmonaire aigu adrénalinique du lapin. C. R. Soc. Biol. (Paris) 151 2, 1957
- 245 Vanquellin & Segales. Quoted from Strelcher 1928.
- 246 Vicaire R. Traité du Cœur p 122, Toulouse 1715 (Quoted from Legallois.)
- 247 Visscher M. B. Haddy F. J. Stephens G. Th. physiology and pharmacology of lung edema. Pharmacol. Rev. 3 389 1956.
- 248 Warthen, H. J. Massive intravenous injections Arch Surg 30 199 1935
- 249 Wasserman A. Loeb L. Mayers n, H. S. Capillary permeability to macromolecules. Circulat. Res. 3 504 1955.
- 250 Weed L. H. McKibben, P. S. Pressure changes in the cerebro-spinal fluid following intravenous injection of solutions of various concentrations. Amer J Physiol. 48 512, 1919
- 251 Welch W. H. Zur Pathologie des Lungenödems. Virchow's Arch. path. Amer 72 373, 1878.
- 252 Werkenhain M. The roentgenological aspect of lung edema. Am J Roentgenol. 41 183, 1939
- 253 Westmark V. Roentgen studies of the lungs and heart. The University of Minnesota Press, Minneapolis 1948.
- 254 Whitehall M. R. Langcope W. T. Williams R. The occurrence and significance of myocardial failure in acute hemorrhagic nephritis. Bull. Johns Hopk. Hosp 61 83, 1939
- 255 Wiggers C. J. Physiology in health and disease p 605, Lea & Febiger Philadelphia 1939
- 256 Wills n, J. G. Pulmonary oedema in acute glomerular nephritis. Arch. Dis. Child. 36 661 1961
- 257 Wood E. H. Moc G. K. The measurement of edema in the heart-lung preparation. Amer J Physiol. 138 506 1942.
- 258 Leonius A., Porte R. R. Swank R. L. Observations on certain manifestations of circulatory congestion produced in dogs by rapid infusion. J. clin. Invest. 22 33, 1943
- 259 Young I. E. Pearce J. E. Stevenson, J. A. Renal responses to hypervolemia in the dog. Canad. J. Biochem. 33 800 1955
- 260 Zaldívar R. i. G. Farinas L. Pulmonar azotico o edema subagudo del pulmón en los renales. Rev. Cuba Cardiol. 14 141 1954.
- 261 Zdanisky E.. Beiträge zur Kenntnis der kardialen Lungentauung auf Grund röntgenologischer klinische und anatomischer Untersuchungen. Wiener Arch. inn. Med. 18 461 1929
- 262 Zdanisky E. Über das Röntgenbild des Lungenödems, ist seine ätiologische Bedeutung zur Frage der Pathogenese des Lungenödems. Röntgenpraxis, 5 248, 1933
- 263 Zettergren, L. Uremic Lung Acta Soc. Med. upsallen 60 161 1935

